© 2015. Published by The Company of Biologists Ltd | The Journal of Experimental Biology (2015) 218, 404-411 doi:10.1242/jeb.110619

RESEARCH ARTICLE

The colony environment modulates sleep in honey bee workers

Ada Eban-Rothschild* and Guy Bloch‡

ABSTRACT

One of the most important and evolutionarily conserved roles of sleep is the processing and consolidation of information acquired during wakefulness. In both insects and mammals, environmental and social stimuli can modify sleep physiology and behavior, yet relatively little is known about the specifics of the wake experiences and their relative contribution to experience-dependent modulation of sleep. Honey bees provide an excellent model system in this regard because their behavioral repertoire is well characterized and the environment they experience during the day can be manipulated while keeping an ecologically and sociobiologically relevant context. We examined whether social experience modulates sleep in honey bees, and evaluated the relative contribution of different social signals. We exposed newly emerged bees to different components of their natural social environment and then monitored their sleep behavior in individual cages in a constant lab environment. We found that rich waking experience modulates subsequent sleep. Bees that experienced the colony environment for 1 or 2 days slept more than same-age sister bees that were caged individually or in small groups in the lab. Furthermore, bees placed in mesh-enclosures in the colony, that prevented direct contact with nestmates, slept similarly to bees freely moving in the colony. These results suggest that social signals that do not require direct or close distance interactions between bees are sufficiently rich to encompass almost the entire effect of the colony on sleep. Our findings provide a remarkable example of social experience-dependent modulation of an essential biological process.

KEY WORDS: *Apis mellifera*, Wake experience, Sleep need, Social environment

INTRODUCTION

The ubiquity of sleep across the animal kingdom and the deterioration in health and performance following sleep deprivation suggest that sleep is a restorative and regenerative biological process essential for survival. Nevertheless, its adaptive value remains elusive (Cirelli and Tononi, 2008; Mignot, 2008). Accumulating evidence suggests that an important and evolutionarily conserved role of sleep is the processing and consolidation of information acquired during wakefulness (Diekelmann and Born, 2010). In both mammals and insects, environmental and social stimuli can modify subsequent sleep physiology and behavior (e.g. Abou-Ismail et al., 2010; Donlea et al., 2009; Ganguly-Fitzgerald et al., 2006; Maquet et al., 2000; Miyamoto et al., 2003), yet only little is known about the specifics of the wake experiences accounting for experience-dependent modulation of sleep.

Department of Ecology, Evolution and Behavior, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, 91904, Israel. *Present address: Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA 94305, USA.

‡Author for correspondence (guy.bloch@mail.huji.ac.il)

Received 9 July 2014; Accepted 4 December 2014

Over the past three decades, sleep or sleep-like states have been described for diverse vertebrate and invertebrate species: fishes (Prober et al., 2006; Yokogawa et al., 2007), insects (Hendricks et al., 2000; Kaiser and Steiner-Kaiser, 1983; Shaw et al., 2000; Tobler, 1983) and even nematode worms (Raizen et al., 2008), in addition to many mammals. Molecular and genetic studies indicate that the molecular pathways associated with sleep in vertebrates and invertebrates show a large degree of conservation. These similarities are consistent with an ancient and common origin for sleep (Allada and Siegel, 2008; Cirelli, 2009). In mammals and birds, sleep is usually defined by typical cortical and muscular activity, whereas in other species sleep is defined by behavioral criteria that include consolidated periods of inactivity associated with reduced muscle tonus, reduced responsiveness to external stimuli, and homeostatic regulation (Hendricks et al., 2000; Tobler, 1983).

In both rodents and flies, environmental and social enrichment modify sleep duration and/or intensity (Gutwein and Fishbein, 1980; Mirmiran et al., 1982; Tagney, 1973). In the fruit fly *Drosophila melanogaster*, social enrichment increases sleep duration and alters sleep architecture (Bushey et al., 2011; Ganguly-Fitzgerald et al., 2006). Sleep duration in socially enriched flies also increases proportionally with the size of the group (Ganguly-Fitzgerald et al., 2006). Given that this increase in sleep is not observed in fly strains with mutations in a subset of short- and long-term memory genes, it has been suggested that the social experience-dependent increase in sleep depends on the ability to form new memories (Donlea et al., 2009; Ganguly-Fitzgerald et al., 2006). In order to better understand experience-dependent modulation of sleep it is necessary to define the wake experiences and environmental signals that modify sleep. Honey bees (*Apis mellifera* Linnaeus 1758) provide an excellent model system with which to study the nature of waking experiences modulating sleep because their behavioral repertoire is well characterized and the environment they experience during the day can be manipulated while keeping an ecologically and sociobiologically relevant context (Eban-Rothschild and Bloch, 2012).

Honey bees are eusocial insects, living in colonies containing a single egg-laying queen, tens of thousands of female workers and a few hundred males (Winston, 1987). Among the workers, there is an age-related division of labor. During the first ~2 weeks of adult life, the workers perform various in-hive activities, such as brood care. Later, worker bees perform various activities in the hive periphery, such as honeycomb construction and nectar storing. From ~3 weeks of adult life, honey bee workers typically forage for pollen and nectar outside the hive (Robinson, 1992; Winston, 1987). The sleep behavior of honey bees has been studied both in the natural context of the colony (Kaiser, 1988; Klein and Seeley, 2011; Klein et al., 2008; Klein et al., 2010) and in detailed laboratory studies (Eban-Rothschild and Bloch, 2008; Kaiser, 1988; Sauer et al., 2003). Honey bees exhibit all three behavioral characteristics of sleep: a period of quiescence (Eban-Rothschild and Bloch, 2008; Kaiser, 1988; Sauer et al., 2003; Sauer et al., 2004), an increased response threshold (Eban-Rothschild and Bloch, 2008; Kaiser, 1988; Kaiser

The Journal of Experimental Biology

The Journal of Experimental Biology (2015) doi:10.1242/jeb.110619

and Steiner-Kaiser, 1983) and a homeostatic regulation mechanism (Klein et al., 2010; Sauer et al., 2004). In honey bee foragers, as in mammals and flies, sleep deprivation impairs learning and memory processes (Beyaert et al., 2012; Hussaini et al., 2009; Klein et al., 2010), and forager bees induced to learn novel navigational tasks sleep longer than control bees (Beyaert et al., 2012).

In this study, we examined the influence of various wake experiences on subsequent sleep in honey bees. We focused specifically on wake experiences that are associated with social interactions in the colony and found evidence for strong environmental modulation of sleep in bees. Interestingly, our results suggest that the colony environment has a strong influence on sleep that does not require close distance interactions with other bees or the brood.

RESULTS

Experiment 1: the influence of the colony environment on subsequent sleep

Forager bees (typically older than 3 weeks of age) that were individually isolated in a constant lab environment illuminated by dim red light were predominantly active during the subjective day and slept during the subjective night (Fig. 1A, Fig. 2B; $P<0.00001$ in all three colonies for the influence of time of day; for the foragers data presented in Fig. 2B, repeated measures one-way ANOVA, d.f.=3, $F_{\text{colony S73}}=23.78$; $F_{\text{colony H1}}=17.98$; $F_{\text{colony H14}}=37.03$). These findings that are based on locomotor activity data are consistent with detailed behavioral observations of sleeping honey bee foragers (Eban-Rothschild and Bloch, 2008; Kaiser, 1988; Klein et al., 2008).

Young bees that experienced the colony environment for their first 1 or 2 days of adult life slept more in the first day of monitoring compared with same-age sister bees that were isolated individually immediately after emergence (Fig. 1B–D, Fig. 2A,B). Furthermore, the differences between the colony-experienced and individually isolated bees were apparent throughout the day, and not restricted to a specific circadian phase (Fig. 2B). An increase in both the number and duration of sleep bouts contributed to the overall increase in total sleep duration in young bees experiencing the colony environment compared with individually isolated bees (Fig. 2C,D). These results demonstrate that a single day of colony experience is sufficient to modulate subsequent sleep in young bees. The differences between foragers and young bees that experience the colony environment suggest that there are additional age- or experience-related changes in sleep.

Experiment 2: the influence of social interactions outside the colony on subsequent sleep

To examine the influences of social interactions on sleep, we confined 30 newly emerged bees for 2 days to a wooden cage outside the colony (‘Lab group’) and later monitored their sleep individually in the laboratory. We found that Lab group bees slept less during the first day in the monitoring cage (Fig. 1E, Fig. 3A,B) and had shorter sleep bouts (Fig. 3C) compared with sister bees experiencing the colony environment for the same period. In two of the trials (with bees from colonies H11 and H12), the Lab group bees appeared to sleep more than individually isolated bees, but these differences were statistically significant only in the trial with colony H12 (Fig. 3A). In a complementary two-way ANOVA that included bees from all three trials, the differences in sleep duration between all three groups were statistically significant (Table 1). Taken together, these results suggest that social interactions with 30 peers outside the colony can modify subsequent sleep, but not to the same extent as the entire colony.

Experiment 3: the influence of direct and indirect interactions with other bees in the colony on subsequent sleep

To uncouple the influence of different components of the colony environment on sleep, we caged 30 newly emerged bees inside a field colony in either single-mesh (SM) or double-mesh (DM) enclosures (enabling or preventing close contact with nestmates outside the enclosure, respectively; see Materials and methods). Bees that during their first 2 days of adult life were allowed to move freely

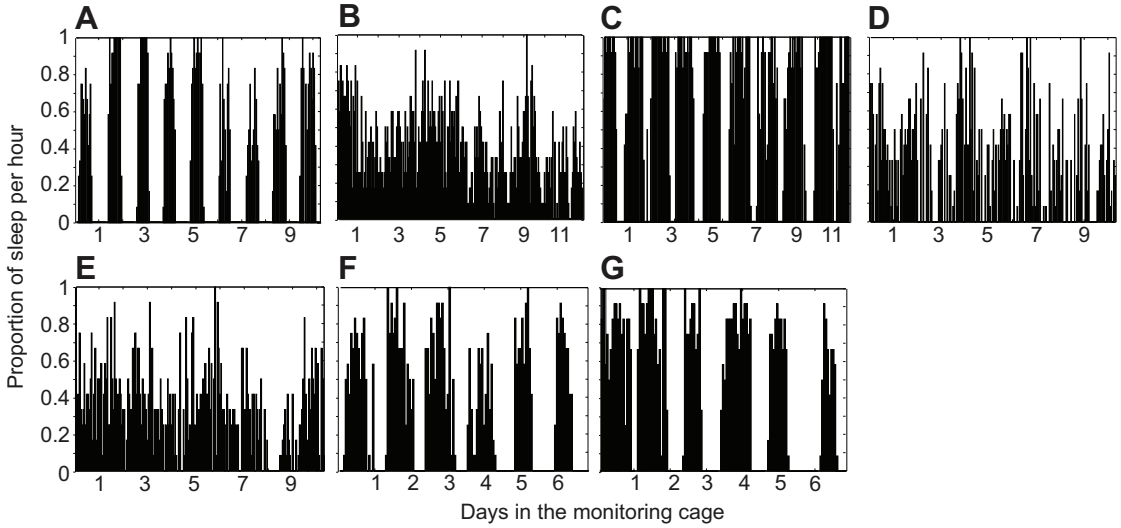

Fig. 1. Representative sleep plots. Each plot depicts the proportion of sleep per hour during the entire monitoring session for an individually isolated honey bee. The experimental chamber was illuminated with constant dim red light. (A) A forager of unknown age (foragers are typically >3 weeks of age). (B,C) A young bee that spent her first 24 h (‘Colony 24 h’, B) or 48 h inside a field colony (‘Colony 48 h’, C). (D) A young bee that spent her first 48 h individually isolated in the lab (‘Lab individually’). (E) A young bee that spent her first 48 h inside a cage containing 30 additional same-age bees in the lab (‘Lab group’). (F,G) A young bee that spent her first 48 h confined with 30 additional same-age bees to a single-mesh (SM) enclosure (‘colony SM’, F) or a double-mesh (DM) enclosure (‘colony DM’, G) inside a colony. Sleep was defined as lack of movement for five consecutive minutes. Differences in the *x*-axis stem from variation in monitoring duration across experiments.

The Journal of Experimental Biology (2015) doi:10.1242/jeb.110619

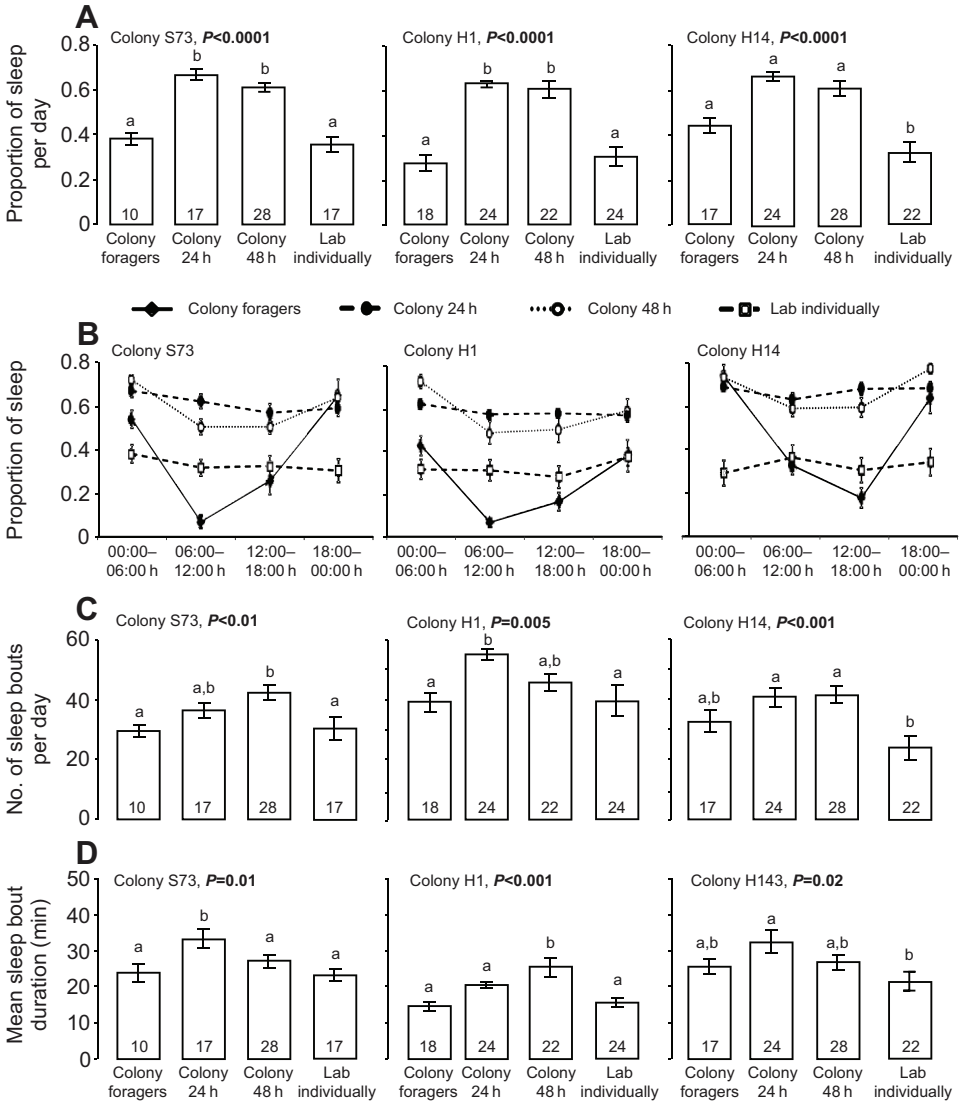

Fig. 2. Exposure to the colony environment influences subsequent sleep in young bees. (A) Proportion of sleep on the first day of isolation in a monitoring cage in a constant lab environment. (B) Proportion of sleep during the first day divided to 6 h bins. (C) Number of sleep bouts during the first day of monitoring. (D) Mean sleep bout duration during the first day of monitoring. Numbers inside bars depict sample size. Each column depicts a repetition with bees from a different source colony. Data are means ± s.e.m. The *P*-values were obtained from one-way ANOVA analyses (bold indicates significance). Groups with different lowercase letters are significantly different in a Tukey's *post hoc* test.

in the colony or were confined to a SM or DM enclosure showed a similar total amount of sleep, number of sleep bouts and mean sleep bout duration (Fig. 1F,G, Fig. 4). However, only in the trial with bees from colony S85 did Lab group bees show a reduced total sleep duration and bout number (Fig. 4A,C) compared with same-age sister bees exposed to the colony. The amount of sleep for the Lab group bees from colonies H2 and HS76 was comparable to that of colony bees and higher than in other experiments with Lab group bees (see Fig. 3). Nevertheless, the mean sleep bout duration of Lab group bees from colonies H2 and HS76 (Fig. 4D) tended to be shorter compared with their sister bees experiencing the colony environment. These findings suggest that social interactions in small groups can have a strong influence on sleep in some genotypes. The similarity between the bees freely moving in the colony, in SM enclosures and in DM enclosures suggests that direct contact with other bees in the colony, the brood or the queen is not necessary for the influence of the colony on sleep.

The influence of time in isolation on subsequent sleep duration

To better understand the influence of experiencing a rich environment on subsequent sleep, we compared sleep duration as a function of time isolated in a monitoring cage in the lab. For this analysis we pooled data across experiments, analyzing together all the bees that experienced the same environment before being isolated in the lab. This analysis shows that the influence of previous social experience was not limited to the first day of monitoring but instead lasted for several days (Fig. 5). Nevertheless, we found a highly significant decrease in the amount of sleep with time in isolation for all groups of bees that experienced a complex social environment prior to monitoring (Fig. 5; $P<0.0001$ for all these groups). Only the bees that were individually isolated shortly after emergence showed no significant changes in the amount of sleep with time in the monitoring cage (Fig. 5, $P=0.7$).

DISCUSSION

Our findings show that social wake experience has profound influences on subsequent sleep in honey bees. Bees that experienced a rich colony environment slept significantly more compared with same-age sister bees that were individually isolated in a poor lab environment. The influence of a rich social experience on sleep persisted for several days after the bees were transferred to individual cages in constant lab conditions. These findings for the honey bee support and extend previous evidence for strong social influences on sleep in *Drosophila* and mammals (Bushey et al., 2011; Ganguly-Fitzgerald et al., 2006; Gutwein and Fishbein, 1980;

The Journal of Experimental Biology (2015) doi:10.1242/jeb.110619

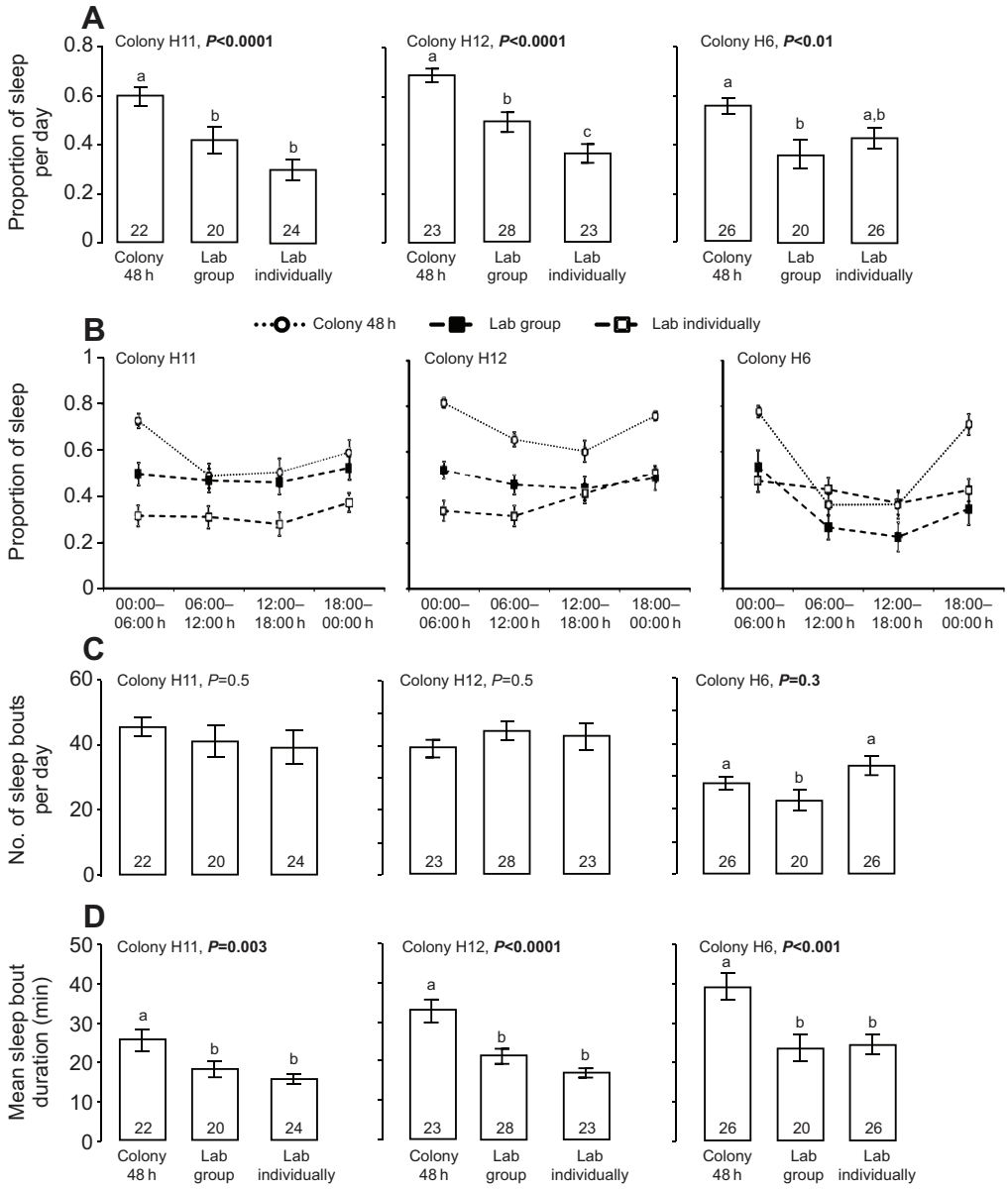

Fig. 3. Social interactions with same-age bees outside the colony lead to an increase in sleep, but not to the same extent as exposure to the entire colony environment. For details of A–D, see Fig. 2. 'Lab group', young bees that were caged in groups of 30 in the lab during the first 48 h after emergence.

Mirmiran et al., 1982; Tagney, 1973). Taking advantage of the honey bee sociobiology, we further suggest that the colony influence on sleep is potent even without direct or close interactions with other bees in the colony. Together, our findings suggest that the social signals that influence sleep include volatile pheromones, sounds, comb vibrations or other environmental variables that are associated with the activity or behavior of other bees in the colony.

Our findings confirm and extend previous evidence for an age-related decrease in honey bee sleep (Eban-Rothschild and Bloch, 2008; Klein et al., 2008; Eban-Rothschild et al., 2012). Young bees slept more than their older sister foragers when the two groups of bees were transferred from the same colony to individual cages in the same lab environment. A similar decrease in sleep duration with age has also been reported for flies (Shaw et al., 2000) and mammals (Dijk et al., 1999; Shaw et al., 2000). The higher sleep requirement in young animals is commonly attributed to the dramatic elaboration of the central nervous system during early life (Kayser et al., 2014). The similarity in age-related changes in sleep is consistent with the premise that many sleep functions are conserved across diverse animal taxa (Allada and Siegel, 2008). Given the importance of sleep at an early age (Kayser et al., 2014), it is interesting to note that our study further shows that the sleep of young honey bees is very sensitive to the social environment they experience during the first 48 h post-pupal emergence.

Young bees that experienced the colony environment slept ~5 h day^{-1} more and had longer and overall more sleep bouts when monitored individually compared with same-age sister bees that were individually isolated shortly after emergence. What in the colony environment modulates sleep? Bees recurrently touch and

Table 1. Two-way ANOVA with multiple comparisons for the data presented in Fig. 3

	d.f.	MS	*F*	*P*
Treatment	2	1.092	28.7	<0.0001
Colony	2	0.09559	2.512	0.0838
Treatment × colony	4	0.05713	1.501	0.2034
Colony 48 h vs Lab group				<0.0001
Colony 48 h vs Lab individually				<0.0001
Lab group vs Lab individually				0.05

The Journal of Experimental Biology (2015) doi:10.1242/jeb.110619

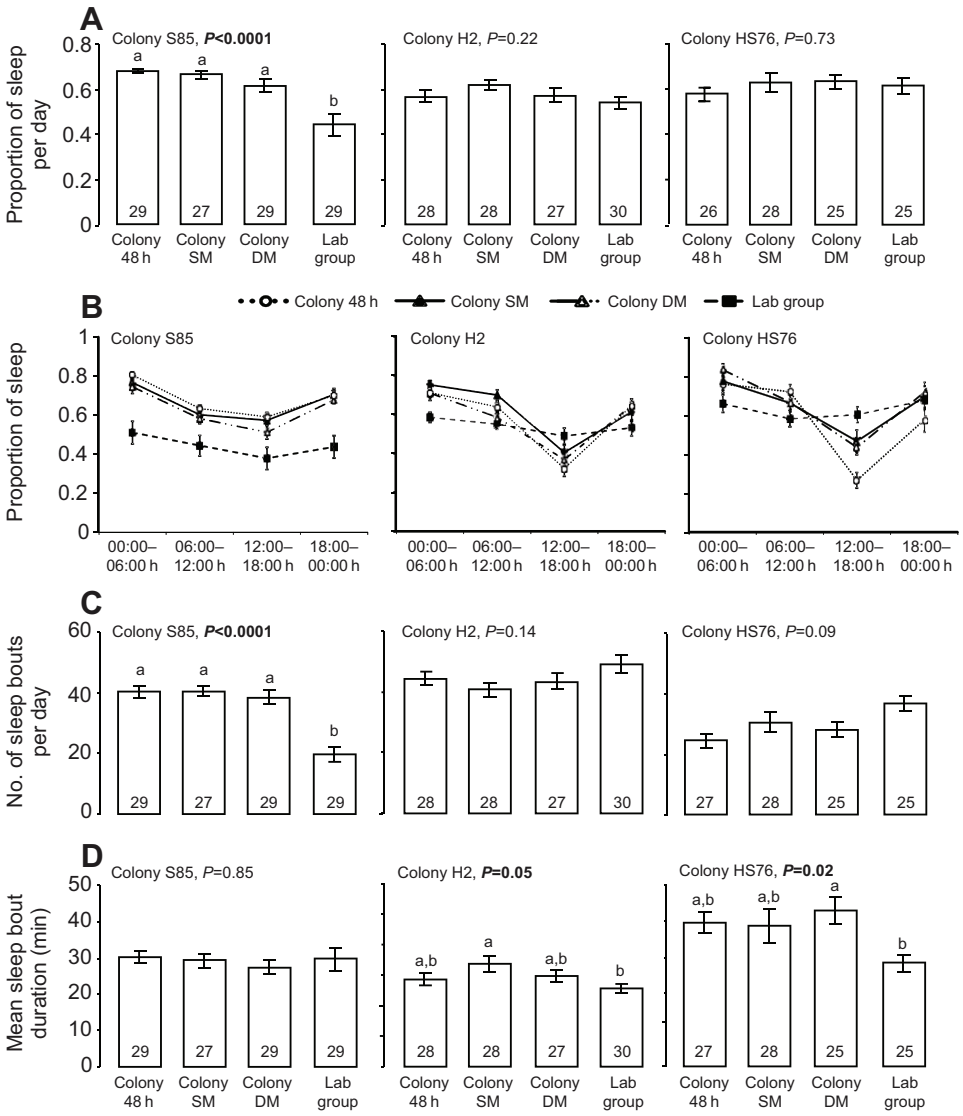

Fig. 4. Direct contact with other bees in the colony is not needed for colony influence on sleep. For details of A–D, see Fig. 2. 'Colony SM', 30 newly emerged bees that were confined to a SM enclosure inside a colony during their first 48 h after emergence; 'Colony DM', same as Colony SM but the enclosure had two meshes.

antennate other bees and these social interactions may affect subsequent sleep. Indeed, in *Drosophila*, sleep duration increased proportionally with the number of flies with which the individual was caged (Ganguly-Fitzgerald et al., 2006; Donlea and Shaw, 2009). Consistent with these findings for the fly, we found that interactions with other individuals also increased subsequent sleep duration in honey bees. Young bees that were housed for 2 days in a poor lab environment together with 30 peers tended to sleep more than same-age bees that were individually isolated in a similar environment. But the natural social environment of honey bees is much more complex than interacting with 30 peers. The honey bee colony is an extremely rich social environment composed of thousands of adult and young individuals releasing numerous chemical, tactile, auditory and vibration signals into the small hive cavity (Winston, 1987). Our findings indeed suggest that experiencing the complexity of the colony led to an even greater increase in subsequent sleep duration (Figs 3, 4).

The specifics and characteristics of the social signals that influence sleep are currently unknown. Using a genetic approach, it was found that mutations in genes involved in vision and olfaction, but not in auditory communication, blocked the increase in sleep following social enrichment in the fruit fly (Ganguly-Fitzgerald et al., 2006). These findings suggest that visual and olfactory social signals are important for social modulation of sleep in *Drosophila*. To start probing the nature of the signals in the colony that influence sleep in honey bees, we used a different approach. We caged focal bees in SM or DM enclosures inside the colony. Both manipulations prevented the bees from moving freely in the colony and performing activities such as brood care or cell cleaning, which are the typical tasks of bees at the tested ages (0–2 days of age). The double mesh also prevented close interactions with other bees in the colony. It is thus remarkable that in three out of three trials, sleep duration and architecture (bout number and duration) did not differ between bees moving freely in the colony and those confined to SM or DM enclosures. In this experiment, in one of the trials, sleep duration was significantly shorter in the Lab group bees compared with the bees that experienced the colony, while in the two other trials, only the mean sleep bout duration was shorter. The lack of difference in total sleep duration in two of the trials differs from the results presented in Fig. 3, and perhaps stems from the high sensitivity of these genotypes to social interactions such that even contact outside of the colony context could modulate their sleep. The finding that the double mesh did not compromise the influence of the colony environment on sleep is intriguing because it suggests that tactile signals, contact pheromones or trophallaxis (i.e. the transfer of food or other fluids among conspecifics), which are all important means

The Journal of Experimental Biology (2015) doi:10.1242/jeb.110619

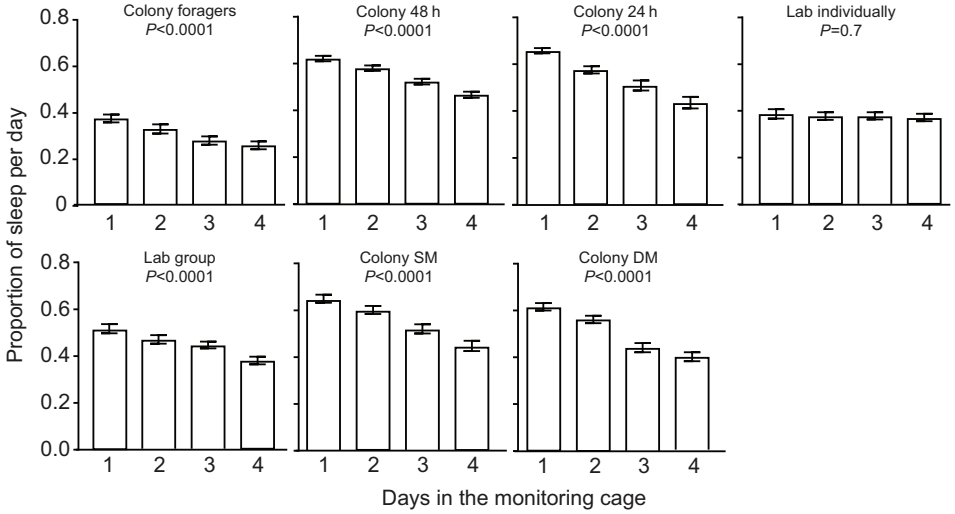

Fig. 5. The experience-mediated influence on sleep duration decreases with time of isolation in a constant lab environment. The plots show the proportion of sleep (means ± s.e.m.) for each day of isolation in a constant lab environment. Each plot summarizes pooled data for all individuals experiencing the specified treatment across experiments. Sample sizes: Colony foragers, *N*=84; Colony 48 h, *N*=213; Colony 24 h, *N*=65; Lab individually, *N*=113; Lab group, *N*=152; Colony SM, *N*=83; Colony DM, *N*=81. *P*-values were obtained from repeated measures ANOVA.

of communication in honey bees, are not necessary for the social experience-dependent modulation of subsequent sleep. These remarkable influences of the colony environment on sleep contrast with a recent study suggesting that in honey bee foragers (which were older than the young bees studied in the current report), sleep duration was not affected by the distance flown between the hive and a food source during the preceding day (Beyaert et al., 2012). In the same study, foragers that were forced to learn new navigation tasks indeed slept longer during the following night, but the differences were around 1 h and there were no differences between bees forced to learn long and short routes (Beyaert et al., 2012). Although the bees and techniques vary significantly between the findings of Beyaert et al. (Beyaert et al., 2012) and our work, the comparison of the two studies suggests the intriguing possibility that social signals can have a stronger influence on honey bee sleep than a cognitively demanding task such as learning a new route. This interesting hypothesis deserves additional research.

Another important question is why do individuals exposed to a rich social environment sleep more than those exposed to a poorer environment? Perhaps bees in the densely populated hive are sleep deprived because they almost continuously need to respond to numerous social signals and frequently interact with their nestmates, and when removed from the colony they compensate by sleeping longer (sleep rebound). This hypothesis is consistent with the finding that sleep duration decreased with time in isolation for all the bees experiencing social interactions but not for the bees individually isolated in a poor lab environment (Fig. 5). However, several studies have suggested that honey bees do not compensate for sleep loss by increasing sleep duration; rather, they suggest that bees recover from sleep deprivation by intensifying their later sleep (Beyaert et al., 2012; Hussaini et al., 2009; Klein et al., 2010; Sauer et al., 2004). Moreover, even if young honey bees were compensating for sleep loss by increasing sleep duration, one would predict that 2 days of sleep deprivation will result in a greater sleep rebound than a single day of sleep deprivation, which is not consistent with our findings that sleep duration was similar for bees experiencing the colony environment for 1 or 2 days (Fig. 2). An alternative explanation for our findings is that bees experiencing the rich colony environment sleep longer than their individually isolated peers because they need more sleep for processing the information they acquired while awake. Several different hypotheses have been raised to explain the association between sleep and cognitive performance, among which is the synaptic homeostasis hypothesis, which predicts a strong association between wake experience and sleep duration (Tononi and Cirelli, 2014). According to this hypothesis, new synapses require both space and energy, and therefore their number must be limited and many are downscaled, mostly during sleep. The synaptic homeostasis hypothesis further predicts that the richer the environment animals are exposed to, the longer is their subsequent sleep. This prediction is supported by studies showing increased synaptic area following a rich wake experience, and a subsequent reduction during sleep (Bushey et al., 2011; Maret et al., 2011). Thus, although the underlying neuronal mechanisms are still elusive, it is possible that the richness of the colony environment entails demanding information processing that leads to an increase in sleep need.

The findings presented above add to the body of evidence on the pervasive influence of the social environment on the behavior and physiology of honey bees (Eban-Rothschild and Bloch, 2012; Ichikawa and Sasaki, 2003; Maleszka et al., 2009). The social manipulations reported here also influenced the development of circadian rhythms in the same young honey bees (Eban-Rothschild et al., 2012). It is thus interesting to note that the influence of the two processes differed to some extent. First, bees experiencing 1 or 2 days in the colony showed a similar increase in sleep but the development of circadian rhythms required 2 days in the colony and a single day was not sufficient for expressing consistent rhythmicity (Eban-Rothschild et al., 2012). Second, confinement with 30 peers in a cage outside the colony led to a significant increase in sleep duration compared with isolation in an individual cage, but did not seem to affect the development of circadian rhythms (Eban-Rothschild et al., 2012). These apparent differences suggest that the influences of the social environment on sleep and on the development of circadian rhythmicity are mediated, at least partially, by different mechanisms. Thus, the same social environment may lead to multiple physiological and behavioral modifications (Eban-Rothschild and Bloch, 2012).

Our findings show that a rich waking experience affects subsequent sleep in young honey bees. The nature of the waking experience is important and social signals, specifically, appear to be pivotal. Additional studies are needed for corroborating our findings suggesting that social information transmitted without direct or close contact encompasses almost the entire influence of the colony environment on sleep and for identifying the most potent social signals that modulate sleep in honey bees.

The Journal of Experimental Biology (2015) doi:10.1242/jeb.110619

MATERIALS AND METHODS

General procedure

Honey bees were derived from a mixture of European races of *A. mellifera* typical to Israel. We kept the colonies according to standard beekeeping techniques in the Bee Research Facility at the Edmond J. Safra campus of the Hebrew University of Jerusalem, Givat-Ram, Jerusalem, Israel. To obtain newly emerged bees, we removed honeycombs containing pupae from source colonies and immediately transferred them to a light-proof container. We placed the container inside an environmental chamber [31±1°C, relative humidity (RH)=55±5%], which was illuminated by constant dim red light. We marked newly emerged bees, under dim red light, with a paint dot on their thorax within 30 min of emergence, and assigned them randomly to one of the test social environments (‘treatments’, see below). After 24 or 48 h, during which the bees experienced the experimental social environments, they were collected and placed individually in a monitoring cage, made from a modified Petri dish (diameter 90 mm, height 15 mm) provisioned with *ad libitum* sugar syrup. On the same day, we also collected sister foragers from the entrance to the source colony (‘Colony foragers’). We identified foragers by pollen loads on their hindlegs, and only collected those with undamaged wings (suggesting relatively little flight experience). In each trial, the bees of all treatment groups originated from the same source colony (‘genotype’). We repeated each experiment three times, each with bees from a different, unrelated colony, in order to establish that our findings were general and not limited to a certain specific genotype (i.e. source colony). The queens of two of the source colonies (S73 and S85) were instrumentally inseminated with semen from a single (different) drone, whereas the other nine colonies were headed by a naturally mated queen (a honey bee queen typically mates with 10–20 drones). Because of the haplodiploid sex-determination system of bees, females from a colony in which the mother queen mated with a single male are closely related (‘full-sisters’) with a coefficient of relationship of R=0.75.

Sleep monitoring

We placed the monitoring cages with the focal bees in an environmental chamber (29±1°C, RH=45±5%), which was illuminated by constant dim red light. We monitored locomotor activity with the ClockLab data acquisition system (Actimetrics Co., Wilmette, IL, USA), four light-sensitive black and white Panasonic WV-BP334 0.08 lx CCD cameras and a high-quality monochrome image acquisition board (IMAQ 1409, National Instruments Co., Austin, TX, USA). Each camera monitored 30 cages simultaneously. Empty cages served as a control for background noise. Data were collected continuously for 4 days, at a frequency of 1 Hz (Shemesh et al., 2007; Shemesh et al., 2010). The findings on the influence of environment on the development of circadian rhythms for these bees were reported previously (Eban-Rothschild et al., 2012). We analyzed sleep behavior using custom-written software (Matlab), based on the data collected by the ClockLab data acquisition system. Previous studies indicated that lack of movement for five consecutive minutes served as a reliable proxy for sleep in honey bees (Beyaert et al., 2012; Eban-Rothschild and Bloch, 2008). Accordingly, we defined sleep as a continuous period lasting 5 min or more during which the monitored bee did not move. A similar index for sleep is routinely used in studies with flies (e.g. Huber et al., 2004; Shaw et al., 2000). For each bee, we calculated the percentage of time asleep, the number of sleep bouts, and the mean sleep bout duration during each day of the experiment.

Statistical analyses

For analyzing the influence of the environment on the various sleep parameters, we used the Prism 6.0 software package (GraphPad Software). We used one-way analysis of variance (ANOVA) tests followed by a Tukey *post hoc* test to compare the proportion of time asleep, mean bout duration and the number of sleep bouts per day. For the data from experiment 2, we performed a complementary two-way ANOVA analysis, because the differences between the three treatments showed a similar trend in two of the colonies, yet the *post hoc* analysis only reached statistical significance in one of them. For the analysis of sleep duration as a function of time in the monitoring cages, we performed repeated measures ANOVA.

Experiment 1: the influence of the colony environment on subsequent sleep

We assigned newly emerged bees to one of the following treatments: (i) 24 h in a field colony (‘Colony 24 h), (ii) 48 h in a field colony (‘Colony 48 h) and (iii) 48 h in an individual cage in the lab (‘Lab individually’). The individual cages were similar to the monitoring cages (see above) but contained pollen, and were kept in a dark environmental chamber (31±1°C, RH=55±5%). We used sister foragers from the same source colony as a positive control (Colony foragers; see above). To compare bees that spent 24 and 48 h in the colony, we marked newly emerged bees during two consecutive days. Between these 2 days the comb with the pupae was placed in a host field colony. Because the bees that were introduced to field colonies were exposed to daylight twice (during the time of introduction and collection from the colony), we also exposed the bees isolated outside the colony to a similar daylight experience. The exposure to light was for less than a minute during the introduction, and for a period lasting 5–10 min at the time of collection. We transferred the bees that were isolated individually to new monitoring cages just before the beginning of the monitoring session. This procedure was followed in order to expose them to a similar handling stress to that experienced by the bees from the other groups that were transferred from the colony to the lab. We repeated this experiment three times, each with bees from a different source colony.

Experiment 2: the influence of social interactions outside the colony on subsequent sleep

We assigned newly emerged bees to the following treatments: (i) 48 h in a field colony (‘Colony 48 h’), (ii) 48 h in a wooden cage (11×10×4.5 cm) with 30 same-age sister bees in the lab (‘Lab group’) and (iii) 48 h in an individual cage in the lab (‘Lab individually’). We placed the individual and group cages in a dark environmental chamber for 48 h (see above). The bees in all treatments experienced similar exposure to daylight (same as above). We repeated this experiment three times, each with bees from a different source colony.

Experiment 3: the influence of direct and indirect interactions with other bees in the colony on subsequent sleep

We assigned newly emerged bees to one of the following treatments: (i) 48 h in a field colony (‘Colony 48 h’), (ii) 48 h in a SM enclosure together with 30 same-age sister bees inside a field colony (‘Colony SM’), (iii) 48 h in a DM enclosure together with 30 same-age sister bees inside a field colony (‘Colony DM’) and (iv) 48 h in a wooden cage (11×10×4.5 cm) with 30 same-age sister bees in the lab (‘Lab group’). The bees in all four treatments experienced similar exposure to daylight (same as above). For the SM treatment, we used a SM enclosure (11×10.5×2 cm) made of an 8-holes per inch mesh that was embedded on an empty comb. For the DM treatment, we surrounded a SM enclosure with a larger 8-holes per inch mesh cage (14×13.5×3 cm), placed 1.5 cm away from the first mesh. Both the SM and DM enclosures prevented the caged bees from interacting with the brood, but allowed exposure to the colony microenvironment (e.g. temperature, humidity and gases such as CO_2), comb vibrations and the colony odors. The DM enclosure, but not the SM, also prevented direct contact with bees outside the enclosure. We placed the frame with the enclosures containing the focal bees in the center of the colony, such that the caged bees were flanked by brood-containing honeycombs. We provisioned the SM and DM cages with *ad libitum* sugar syrup and pollen, similar to the bees caged outside the colony (see above). The density of bees inside the SM and DM enclosures was similar to that in wooden cages in the lab. Prior to the experiment (before introducing the focal bees), we placed the empty honeycomb frames with the SM and DM enclosures inside the source colonies for 2–3 days. This was done in order to allow the honeycomb to absorb colony odors and minimize confounding factors associated with exposing bees from the different treatment groups to different honeycombs. During this period, and in all the trials of this experiment, there were no eggs laid on the honeycombs into which we embedded the mesh enclosures. We repeated this experiment three times, each with bees from a different source colony.

Acknowledgements
We thank Yair Shemesh for discussions and help with the experiments; Rafi Nir, Yafit Brenner and Nadav Yayon for assistance with the bees; and Ron Katzir for preparing and validating the sleep analysis software.

Competing interests
The authors declare no competing or financial interests.

Author contributions
A.E.R. and G.B. designed the study, A.E.R. performed the experiments and analyzed the results, A.E.R. and G.B. wrote and revised the article.

Funding
This work was funded by the Israel Science Foundation (ISF, grant number 1662/11 to G.B.) and the US–Israel Binational Science Foundation (BSF, grant number 2007465 to G.B.).

References

Abou-Ismail, U. A., Burman, O. H., Nicol, C. J. and Mendl, M. (2010). The effects of enhancing cage complexity on the behaviour and welfare of laboratory rats. *Behav. Processes* **85**, 172-180.
Allada, R. and Siegel, J. M. (2008). Unearthing the phylogenetic roots of sleep. *Curr. Biol.* **18**, R670-R679.
Beyaert, L., Greggers, U. and Menzel, R. (2012). Honeybees consolidate navigation memory during sleep. *J. Exp. Biol.* **215**, 3981-3988.
Bushey, D., Tononi, G. and Cirelli, C. (2011). Sleep and synaptic homeostasis: structural evidence in *Drosophila*. *Science* **332**, 1576-1581.
Cirelli, C. (2009). The genetic and molecular regulation of sleep: from fruit flies to humans. *Nat. Rev. Neurosci.* **10**, 549-560.
Cirelli, C. and Tononi, G. (2008). Is sleep essential? *PLoS Biol.* **6**, e216.
Diekelmann, S. and Born, J. (2010). The memory function of sleep. *Nat. Rev. Neurosci.* **11**, 114-126.
Dijk, D. J., Duffy, J. F., Riel, E., Shanahan, T. L. and Czeisler, C. A. (1999). Ageing and the circadian and homeostatic regulation of human sleep during forced desynchrony of rest, melatonin and temperature rhythms. *J. Physiol.* **516**, 611-627.
Donlea, J. M. and Shaw, P. J. (2009). Sleeping together using social interactions to understand the role of sleep in plasticity. *Adv. Genet.* **68**, 57-81.
Donlea, J. M., Ramanan, N. and Shaw, P. J. (2009). Use-dependent plasticity in clock neurons regulates sleep need in *Drosophila*. *Science* **324**, 105-108.
Eban-Rothschild, A. D. and Bloch, G. (2008). Differences in the sleep architecture of forager and young honeybees (*Apis mellifera*). *J. Exp. Biol.* **211**, 2408-2416.
Eban-Rothschild, A. and Bloch, G. (2012). Social influences on circadian rhythms and sleep in insects. *Adv. Genet.* **77**, 1-32.
Eban-Rothschild, A., Shemesh, Y. and Bloch, G. (2012). The colony environment, but not direct contact with conspecifics, influences the development of circadian rhythms in honey bees. *J. Biol. Rhythms* **27**, 217-225.
Ganguly-Fitzgerald, I., Donlea, J. and Shaw, P. J. (2006). Waking experience affects sleep need in *Drosophila*. *Science* **313**, 1775-1781.
Gutwein, B. M. and Fishbein, W. (1980). Paradoxical sleep and memory (I): selective alterations following enriched and impoverished environmental rearing. *Brain Res. Bull.* **5**, 9-12.
Hendricks, J. C., Finn, S. M., Panckeri, K. A., Chavkin, J., Williams, J. A., Sehgal, A. and Pack, A. I. (2000). Rest in *Drosophila* is a sleep-like state. *Neuron* **25**, 129-138.
Huber, R., Hill, S. L., Holladay, C., Biesiadecki, M., Tononi, G. and Cirelli, C. (2004). Sleep homeostasis in *Drosophila melanogaster*. *Sleep* **27**, 628-639.
Hussaini, S. A., Bogusch, L., Landgraf, T. and Menzel, R. (2009). Sleep deprivation affects extinction but not acquisition memory in honeybees. *Learn. Mem.* **16**, 698-705.
Ichikawa, N. and Sasaki, M. (2003). Importance of social stimuli for the development of learning capability in honeybees. *Appl. Entomol. Zool. (Jpn.)* **38**, 203-209.
Kaiser, W. (1988). Busy bees need rest, too: Behavioral and electromyographical sleep signs in honeybees. *J. Comp. Physiol. A* **163**, 565-584.
Kaiser, W. and Steiner-Kaiser, J. (1983). Neuronal correlates of sleep, wakefulness and arousal in a diurnal insect. *Nature* **301**, 707-709.
Kayser, M. S., Yue, Z. and Sehgal, A. (2014). A critical period of sleep for development of courtship circuitry and behavior in *Drosophila*. *Science* **344**, 269-274.
Klein, B. A. and Seeley, T. D. (2011). Work or sleep? Honeybee foragers opportunistically nap during the day when forage is not available. *Anim. Behav.* **82**, 77-83.
Klein, B. A., Olzsowy, K. M., Klein, A., Saunders, K. M. and Seeley, T. D. (2008). Caste-dependent sleep of worker honey bees. *J. Exp. Biol.* **211**, 3028-3040.
Klein, B. A., Klein, A., Wray, M. K., Mueller, U. G. and Seeley, T. D. (2010). Sleep deprivation impairs precision of waggle dance signaling in honey bees. *Proc. Natl. Acad. Sci. USA* **107**, 22705-22709.
Maleszka, J., Barron, A. B., Helliwell, P. G. and Maleszka, R. (2009). Effect of age, behaviour and social environment on honey bee brain plasticity. *J. Comp. Physiol. A* **195**, 733-740.
Maquet, P., Laureys, S., Peigneux, P., Fuchs, S., Petiau, C., Phillips, C., Aerts, J., Del Fiore, G., Degueldre, C., Meulemans, T. et al. (2000). Experience-dependent changes in cerebral activation during human REM sleep. *Nat. Neurosci.* **3**, 831-836.
Maret, S., Faraguna, U., Nelson, A. B., Cirelli, C. and Tononi, G. (2011). Sleep and waking modulate spine turnover in the adolescent mouse cortex. *Nat. Neurosci.* **14**, 1418-1420.
Mignot, E. (2008). Why we sleep: the temporal organization of recovery. *PLoS Biol.* **6**, e106.
Mirmiran, M., van den Dungen, H. and Uylings, H. B. (1982). Sleep patterns during rearing under different environmental conditions in juvenile rats. *Brain Res.* **233**, 287-298.
Miyamoto, H., Katagiri, H. and Hensch, T. (2003). Experience-dependent slow-wave sleep development. *Nat. Neurosci.* **6**, 553-554.
Prober, D. A., Rihel, J., Onah, A. A., Sung, R. J. and Schier, A. F. (2006). Hypocretin/orexin overexpression induces an insomnia-like phenotype in zebrafish. *J. Neurosci.* **26**, 13400-13410.
Raizen, D. M., Zimmerman, J. E., Maycock, M. H., Ta, U. D., You, Y. J., Sundaram, M. V. and Pack, A. I. (2008). Lethargus is a *Caenorhabditis elegans* sleep-like state. *Nature* **451**, 569-572.
Robinson, G. E. (1992). Regulation of division of labor in insect societies. *Annu. Rev. Entomol.* **37**, 637-665.
Sauer, S., Kinkelin, M., Herrmann, E. and Kaiser, W. (2003). The dynamics of sleep-like behaviour in honey bees. *J. Comp. Physiol. A* **189**, 599-607.
Sauer, S., Herrmann, E. and Kaiser, W. (2004). Sleep deprivation in honey bees. *J. Sleep Res.* **13**, 145-152.
Shaw, P. J., Cirelli, C., Greenspan, R. J. and Tononi, G. (2000). Correlates of sleep and waking in *Drosophila melanogaster*. *Science* **287**, 1834-1837.
Shemesh, Y., Cohen, M. and Bloch, G. (2007). Natural plasticity in circadian rhythms is mediated by reorganization in the molecular clockwork in honeybees. *FASEB J.* **21**, 2304-2311.
Shemesh, Y., Eban-Rothschild, A., Cohen, M. and Bloch, G. (2010). Molecular dynamics and social regulation of context-dependent plasticity in the circadian clockwork of the honey bee. *J. Neurosci.* **30**, 12517-12525.
Tagney, J. (1973). Sleep patterns related to rearing rats in enriched and impoverished environments. *Brain Res.* **53**, 353-361.
Tobler, I. (1983). Effect of forced locomotion on the rest-activity cycle of the cockroach. *Behav. Brain Res.* **8**, 351-360.
Tononi, G. and Cirelli, C. (2014). Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration. *Neuron* **81**, 12-34.
Winston, M. L. (1987). *The Biology of the Honey Bee*. Cambridge, MA: Harvard University Press.
Yokogawa, T., Marin, W., Faraco, J., Pézeron, G., Appelbaum, L., Zhang, J., Rosa, F., Mourrain, P. and Mignot, E. (2007). Characterization of sleep in zebrafish and insomnia in hypocretin receptor mutants. *PLoS Biol.* **5**, e277.