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Caste-dependent sleep of worker honey bees

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SUMMARY

Sleep is a dynamic phenomenon that changes throughout an organism's lifetime, relating to possible age- or task-associated changes in health, learning ability, vigilance and fitness. Sleep has been identified experimentally in many animals, including honey bees (*Apis mellifera*). As worker bees age they change castes, typically performing a sequence of different task sets (as 'cell cleaners', 'nurse bees', 'food storers' and 'foragers'). Belonging to a caste could differentially impact the duration, constitution and periodicity of a bee's sleep. We observed individually marked bees within observation hives to determine caste-dependent patterns of sleep behavior. We conducted three studies to investigate the duration and periodicity of sleep when bees were outside comb cells, as well as duration of *potential* sleep when bees were immobile inside cells. All four worker castes we examined exhibited a sleep state. As bees aged and changed tasks, however, they spent more time and longer uninterrupted periods in a sleep state outside cells, but spent less time and shorter uninterrupted periods immobile inside cells. Although cell cleaners and nurse bees exhibited no sleep:wake rhythmicity, food storers and foragers experienced a 24 h sleep:wake cycle, with more sleep and longer unbroken bouts of sleep during the night than during the day. If immobility within cells is an indicator of sleep, our study reveals that the youngest adult bees sleep the most, with all older castes sleeping the same amount. This in-cell potential sleep may compensate for what would otherwise indicate an exceptional increase of sleep in an aging animal.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/211/18/3028/DC1

Key words: circadian rhythms, division of labor, ontogeny, rest, sleep, worker honey bees.

INTRODUCTION

Sleep is a flexible behavior that can change in duration, constitution and periodicity throughout an organism's lifetime. Duration, constitution and periodicity of sleep depend on many factors, including age (Roffwarg et al., 1966; Jenni et al., 2005). Humans, for example, exhibit far more rapid eye movement (REM) sleep and sleep in general, but less circadian organization to the timing of sleep during infancy than during adolescence or maturity. The same pattern of reduced sleep with increasing age exists in rats, cats and guinea pigs (Jouvet-Mounier et al., 1970) and is generally thought to exist in all mammals (Frank and Heller, 2003).

Sleep ontogeny has also been investigated in two invertebrate species, the fruit fly *Drosophila melanogaster* Meigen 1830, and the honey bee *Apis mellifera* Linnaeus 1758. Fruit flies, like mammals, sleep less as they age (Shaw et al., 2000). Periods of sleep and wake become less consolidated in aging fruit flies, but not in aging humans (except for the elderly) (Koh et al., 2006). As for honey bees, data on the ontogeny of sleep duration and periodicity are limited to work conducted on rest:activity rhythms measured in isolated laboratory individuals (Spangler, 1972; Sauer et al., 1998; Sauer et al., 1999; Toma et al., 2000) and ambiguous measures of sleep of workers within the hive (Lindauer, 1952; Moore et al., 1998).

Definition of sleep in honey bees

Although the set of characters considered diagnostic of sleep ranges widely and no set of characters has been universally adopted in the literature, several 'sleep signs' are deemed critical by most researchers when defining sleep behaviorally. A sleeping organism exhibits a specific *posture* during *easily reversible* bouts of *relative immobility*, during which its *arousal threshold is increased* (Flanigan, 1972). According to Tobler (Tobler, 1985), such a state should be *internally controlled*. The definition of sleep, initially behavioral, expanded to include correlative electrophysiological measures and the combination of behavior and electrophysiological recordings has often been used to identify sleep in vertebrates (Flanigan et al., 1973). Individually, behavior and electrophysiology present limitations when used to define sleep. Brain states often differ between sleeping and wakeful organisms, but relying on electrophysiology alone can result in misidentification of sleep in mammals and birds, and is less informative for other animals (Campbell and Tobler, 1984). Some attempts to electrophysiologically distinguish between wakeful and quiescent states in invertebrates have been performed (Kaiser and Steiner-Kaiser, 1983; Schuppe, 1995, Nitz et al., 2002; Ramón et al., 2004), but these gross measures require coincident behavioral characters to reliably establish sleep. Alternatively, relying exclusively on a subset of behavioral characters can also be misleading. Immobile animals can have low arousal thresholds, and animals with high arousal thresholds can be awake but reluctant to move (e.g. habituated to disturbance). A cautious application of operational definitions relying on correlations of sleep signs is often a necessity when identifying a sleeping animal.

Versions of the behavioral definition of sleep have been measured and reported in many vertebrate species and in a handful of

invertebrate species (Rattenborg and Amlaner, 2002). Behavioral sleep has been identified in a cuttlefish (Duntley and Morrissey, 2004), an octopus (Brown et al., 2006), a crayfish (Ramón et al., 2004), species of scorpions (Tobler and Stalder, 1988), cockroaches (Tobler, 1983; Tobler and Neuner-Jehle, 1992) and a paper wasp (Klein, 2003). The most extensive invertebrate sleep research has been conducted with the fruit fly *D. melanogaster* (Hendricks et al., 2000; Shaw et al., 2000) and the honey bee *A. mellifera* (Kaiser, 1988; Sauer et al., 2004).

Apis mellifera workers exhibit age polyethism, or the changing of task sets with age. A worker honey bee begins life as an egg laid within a beeswax cell. After passing through the developmental stages of larva and pupa, the freshly eclosed worker (called a callow) spends the first days of her adult life as a member of the 'cell cleaner' caste, spending much of her time oriented headfirst in cells – occasionally cleaning these cells (Seeley, 1982; Seeley and Kolmes, 1991; Moore, 2001). After 3 days as a cell cleaner, the typical worker spends days 4–12 of adulthood as a 'nurse bee', feeding and tending brood and the queen, followed by days 13–20 as a 'food storer' (or 'middle-aged bee'), receiving and storing fresh nectar (Seeley, 1982; Johnson, 2008). A worker bee spends her remaining days in the 'forager' caste, exiting the hive in the search for and acquisition of nectar and pollen to feed her colony. The age polyethism schedule of worker honey bees is flexible, and depends on variables ranging from genetic predisposition (Calderone and Page, 1989) to colony needs (Seeley, 1995) and the caste demographics within a colony (Huang and Robinson, 1996). Task sets performed by bees may profoundly influence bee sleep, as demonstrated when Bloch and Robinson (Bloch and Robinson, 2001) induced foragers to perform the tasks of nurse bees, resulting in a reversion from rhythmic back to arrhythmic behavior.

Kaiser and Steiner-Kaiser (Kaiser and Steiner-Kaiser, 1983) first discovered the potential for sleep in *A. mellifera* by tethering isolated foragers under constant light conditions and recording circadian sensitivity of optomotor interneurons to moving visual stimuli. Kaiser (Kaiser, 1988) and Sauer et al. (Sauer et al., 2004) followed this electrophysiological work by performing a series of meticulous studies on isolated foragers that examined behavioral and physiological characters associated with sleep. Having satisfied the criteria defined above as diagnostic of sleep, worker honey bees appear to be sleeping when relatively immobile, with body and appendages slumping in the direction of gravity (Fig. 1) [for more descriptions, see Kaiser (Kaiser, 1988)]. 'Relative immobility' refers here to bees that are immobile except for exhibiting occasional, apparently spontaneous antennal, tarsal or leg twitches or proboscis extensions, or discontinuous respiratory pumping motions of the gaster (posterior body tagma of hymenopterans). This postural state can be sustained for extended periods, but is easily reversed, often by the physical contact of a neighboring bee (see Movie 1 in supplementary material).

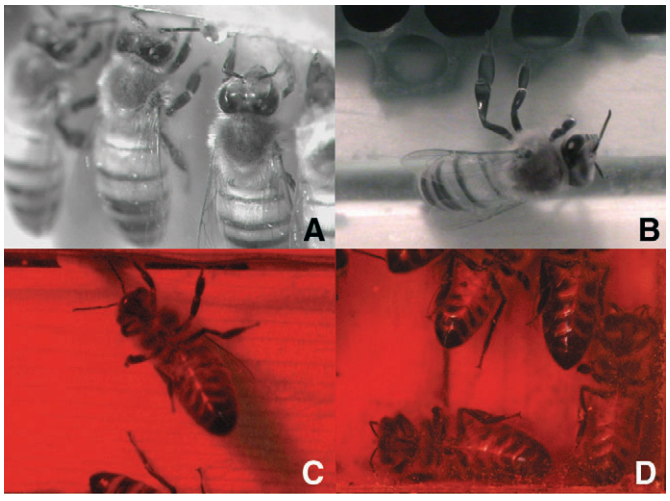

Fig. 1. Worker honey bees displaying typical sleep postures while relatively immobile, with limbs and body drooping in the direction of gravity. Bees exhibit a sleep state while (A) in groups, (B) isolated, (C) dangling motionless from tarsal claws, or (D) leaning against the observation hive wall or floor. Photographs taken with a Panasonic AG-DVC30 video camera in infrared-sensitive mode (A,B) and a Nikon D70 under red lamplight (C,D).

With a definition of sleep and an understanding of the typical sequence of age-correlated behaviors in worker honey bees, we can pose questions about how sleep duration, constitution and periodicity change with respect to caste and age. How long do members of each caste sleep, when do they sleep, and at what point in an adult bee's lifetime do sleep:wake rhythms take shape? Worker bees have been the subject of numerous studies regarding circadian rhythmicity, some that explicitly relate to sleep (Kaiser, 1988; Sauer and Kaiser, 1995; Sauer et al., 1998; Sauer et al., 1999; Bloch and Robinson, 2001), but many more that ambiguously address sleep biology, either by examining 'presumptive inactive behaviors' (Moore et al., 1998) or by examining circadian *activity* rhythms (Spangler, 1972; Southwick and Moritz, 1987; Toma et al., 2000; Meshi and Bloch, 2007).

Honey bee foragers follow very strong diurnal rhythms of activity, including visiting flowers and dance communication (von Frisch, 1967). Lindauer (Lindauer, 1952) observed one pollen forager continuously for 2 days and, although he did not distinguish sleep from a state of being 'Müßig' (idle), he found that the forager rested more at night than during the day (although grooming was included in this measure). Kaiser (Kaiser, 1988) followed up on this observation with extensive studies that consistently supported a circadian sleep:wake cycle of experimentally isolated foragers. Sauer and Kaiser (Sauer and Kaiser, 1995) confirmed that pollen foragers also exhibit circadian rest within an observation hive. The younger nurse bees, in contrast, do not show diel rest–activity rhythms (Lindauer, 1952; Crailsheim et al., 1996; Moore et al., 1998), although they do show non-circadian sleep signs (Sauer et al., 1999). Spangler (Spangler, 1972) reported no circadian activity in a freshly eclosed and isolated worker and contrasted this with an older worker, which exhibited circadian activity cycles. Moore et al. (Moore et al., 1998) recorded various in-hive tasks, all of which were performed arrhythmically except 'resting', which increased at night relative to the day as workers aged. Sauer et al. (Sauer et al., 1998; Sauer et al. 1999) specifically examined sleep signs within developing adults living in isolation and found an increasing circadian organization to the timing of sleep as the bees aged.

Our aim is to determine whether or not worker bees living in a natural colony setting engage in more rhythmic and shorter sleep as they grow older. To do so, we observed worker honey bees for caste-dependent sleep behavior, recording duration and periodicity of sleep when bees were outside comb cells, and immobility when bees were inside cells. Some preliminary results have been previously presented as an abstract (Klein, 2006).

MATERIALS AND METHODS

Our data were obtained from three studies on two colonies, featuring scan sampling and focal sampling of subjects categorized by

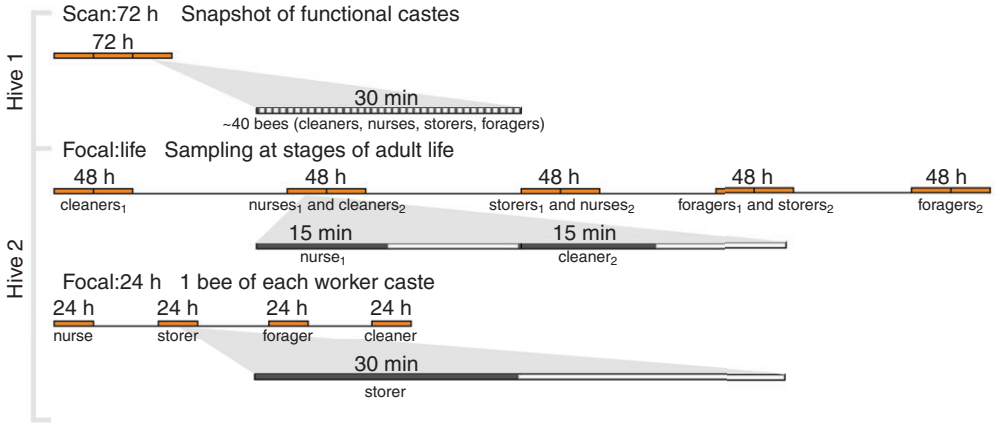

Fig. 2. Timeline of each sampling method. (Scan:72 h) Scan sampling of behavior of ~40 worker bees every 30 min for 72 h. Enlarged view represents one of 144 consecutive 30 min periods, and each dark bar signifies a single observation of one of the ~40 bees. Bees were selected by the function they performed (or by evidence of their recent eclosion, in the case of cell cleaners) prior to the 72 h study. (Focal:life) Focal sampling of worker bees at important stages of their adult lives. Two groups of cell cleaners were introduced (represented by the subscripts 1 and 2) and two randomly selected bees were each observed for 15 min each hour (enlarged view) for 48 h periods. (Focal:24 h) Focal sampling of a representative of each worker caste. Each bee was observed for 30 min every hour for 24 h. Enlarged view represents 1 h of 24 consecutive hours during which a single food storer was observed for 30 min.

behavior or known age. We chose different sampling methods in order to balance the tradeoff between obtaining a relatively complete picture of each bee and a representative picture of each caste. We scan-sampled bees in one hive, recording behavior for 3 days (Scan:72 h), we focal-sampled bees selected pseudo-randomly within a known age group in a second colony for periods throughout their adult lives (Focal:life), and we selected one representative of each age caste from this second colony and recorded her behavior for 1 day (Focal:24 h); see details below (Fig. 2).

We conducted separate studies to control for colony differences and environmental fluctuations while obtaining information that was representative of worker bee sleep behavior. Scan sampling and focal sampling honey bee behavior have been shown to produce 'indistinguishable' results (Kolmes, 1984). Using both methods enabled us to control for environmental fluctuations by condensing our observations into a 3 day snapshot or extending our observations across adult lifespans. Furthermore, we introduced cell cleaners twice during the Focal:life study, allowing us to examine the behavior of each age caste within the same colony on different dates. This, along with conducting the Scan:72 h study at a different time and with a different colony than the Focal:life study, decreased the possibility that the behavior patterns we found were idiosyncratic, a function of the particular colonies, or a result of weather conditions during our study.

Experimental design

We set up two two-frame observation hives (Seeley, 1995), each hive containing approximately 2000 New World Carniolan honey bees (*Apis mellifera carnica*; queen breeder: C. F. Koehnen and Sons, Inc., Glenn, CA, USA) on 23 and 25 June 2005 at Cornell University's Liddell Field Station (Ithaca, New York, USA). We placed the two observation hives in separate rooms of the field station, gave the bees free access to the outdoors, and restricted each colony's queen to the bottom frame by inserting a queen-excluder between the two frames. Bees collected food primarily from wild flowers surrounding the field station.

B.A.K. and K.M.O. observed adult worker honey bees labeled for individual identification and belonging to different age or functional castes for 72 h (hive 1), and 48 and 24 h (hive 2) durations between 17 July and 15 August 2005 under ambient light (daytime) or red light (nighttime), to which honey bees are less visually sensitive (von Frisch, 1967). Red light shone from a lamp mounted to each side of the observation hives and these lamps remained on for the duration of each study. After we recorded a bee's behavior, we shone a handheld LED light when necessary to clarify the identity of the marked bee. The sun was visible at approx. 05:45 h and the sun set at approx. 20:15–20:30 h, so for the purposes of this study daytime is defined as 06:00–20:00 h and nighttime as 20:00–06:00 h and 'periodicity' refers to the day *versus* night presence–absence of sleep behaviors. Indoor temperature remained between 22–25.5°C. Ambient sunlight probably did not strike the inside of our observation hives *via* the hive entrance, but indirectly shone through a window that was perpendicular to the plane of each observation hive. Levels of indirect light changed during the course of each day, but curtains prevented any direct sunlight from striking either hive. *Apis mellifera* have been known to colonize sites exposed to ambient light, although this behavior is not common and effects of ambient light on diurnal sleep patterns are unknown.

Marking

We marked bees with unique combinations of color, repeated on the dorsal and ventral sides of the gaster to facilitate identification of a bee when she was in a cell or dorsally obscured (e.g. while clinging to the glass pane of hive). We repeated the color combinations on the dorsal alitrunk (midsection of hymenopteran body; Scan:72 h study), or attached numbered plastic labels with Canada balsam (Focal:life and Focal:24 h studies). We chilled bees prior to marking, except in the case of callows, which are more sensitive to such treatment. The marked bees acclimatized for 2.5–3 h (1 h for foragers) within holding cages adjacent to the observation hives, then spent 2–7 h (3 h for Scan:72 h study, 7 h and 2.5 h for Focal:life study, and 2 h for callow in Focal:24 h study) within the hives prior to behavioral recordings.

Markers: shellac plus dry artists' pigments for Scan:72 h study, or shellac plus dry artists' pigments combined with Sharpie oil-based marker, Prismacolor marker or Pentel correction 'Presto!' for Focal:life and Focal:24 h studies.

Behaviors recorded

For bees in a relaxed state, we recorded three forms of relative immobility (bee without motion except for spontaneous leg or tarsal twitching, proboscis extension or respiratory pumping of gaster): (1) with antennae immobile; (2) with minute twitching of antennae; or (3) with larger, usually swaying motions of antennae (see Movies 2–4 in supplementary material, respectively). We also recorded whether each relaxed, relatively immobile bee was inside or outside a cell. When inside a cell, a bee's antennae were not visible, rendering the three forms of relative immobility indistinguishable.

We distinguished the relative immobility of relaxed bees, just described, from the active state (bee locomotes, grooms, lifts or turns body or fans wings), and from the relative immobility of non-relaxed bees (bee exhibits an alert stance, is groomed by another bee, antennates rapidly between bouts of locomotion, lifts a leg, turns her head, engages in trophallaxis, or processes wax with her mandibles). Movements associated with wakefulness while in cells include turning or rhythmic motions of the body (Sakagami, 1953), or continuous pumping of the gaster, including when bees are heating adjacent brood cells (Kleinhenz et al., 2003).

Scan:72 h

We collected and marked four sets of 13 adult workers, presumed to belong to the four worker castes: cell cleaners from the original source colony, nurse bees within the observation hive's brood comb cells, food storers from within the upper frame's (brood-free) cells, and foragers netted prior to re-entry into the observation hive (hive 1). Following a predetermined path, we visually scanned the hive's frames for marked bees and observed each marked bee for 3–5 s to determine its behavioral state. If the bee was immobile but jostled by another bee during this time, we waited 5 s and observed the bee again. We recorded the behavior of each visible, marked bee every 30 min for 72 h (3–5 s per bee × ~40 bees/30 min × 72 h; Fig. 2).

Focal:life

We introduced 30 and 40 recently eclosed adult bees (callows) to hive 2 on 17th and 23rd July, respectively, and began recording the callows' behavior 14 h and 9 h after they had been collected (7 h and 2.5 h after introduction into hive, respectively). We selected two of these bees per hour (from a non-repeating pseudo-randomly generated list of numbers) and recorded the behavior of each bee continuously for 15 min. We made continuous, real-time recordings by typing keys on a laptop as we observed behaviors, each key representing a different behavior, using JWatcher 0.9, a freeware behavior-recording and analysis program (Blumstein et al., 2000). We repeated this recording of bees' behavior for 15 min periods every hour, two bees per hour, for 48 h. Each week, as the bees aged and changed tasks, we repeated this procedure (15 min per bee × 2 bees per hour × 48 h per week × 4 weeks; i.e. 48 h on, 4–8 days off, 48 h on, etc.; Fig. 2). We began our 48 h study periods on July 17th, 23rd, 29th and August 8th and 13th.

Focal:24 h

We selected four bees from hive 2, one bee representing each worker caste. We recorded the behavior of one bee continuously for 30 min each hour for 24 h (30 min per hour × 1 bee of each worker caste × 24 h) and repeated this recording regimen for each of the other three bees on separate days (Fig. 2). Continuous recordings were again made using JWatcher 0.9. Of the four bees examined, the nurse bee, food storer and forager were selected from among the subjects used in the Focal:life study and the cell cleaner was newly marked and reintroduced for this study, conducted on August 3rd, 7th, 10th and 13th, respectively. We drew maps showing cell visits by the cell cleaner and by the food storer that lasted longer than several seconds, as well as sleep sites of the forager, recorded within the hourly 30 min observation periods.

Analysis

We conducted one-way *F*-tests for every analysis distinguishing day *versus* night behavior and two-way *F*-tests for every analysis with the additional factor of worker caste. We followed up statistically significant results by making pairwise comparisons using the Tukey–Kramer HSD test, or, when interactions were analyzed, we decomposed significant interactions using a simple main effects test ('Test Slices'). We treated multiple observations on the same individual as independent observations. We conducted an additional analysis of results from the Focal:life study eliminating all data except for one observation per bee per caste to address the assumption of independence of data. We report summary statistics of continuous variables as means ± standard error (s.e.m.). We set alpha at 0.05 for all tests and all tests were two-tailed. Owing to the non-normal distribution of our data, we also analyzed behavioral data (without interactions) using nonparametric tests. We analyzed data with two levels (day *vs* night) using the Wilcoxon test and four levels (worker castes) using the Kruskal–Wallis test. We analyzed all data with the JMP IN (version 5.1.2; SAS Institute Inc. 2004) computer package and we conducted all analyses on a Windows XP machine with a Pentium-IV processor.

A.K. tested for rhythmicity of behaviors in the Scan:72h data by using integrated analytical tools (Flytoolbox) developed by Levine et al. (Levine et al.. 2002) using MATLAB (version 7.4; The MathWorks 2007). A.K. plotted correlograms using the simple signal processing functions of Flytoolbox to nonlinearly detrend the data with a 72 h high-pass Butterworth filter while applying the autocorrelation function. Levine et al. (Levine et al., 2002) discuss these methods and the precedence and value of using the autocorrelation function to assess rhythmicity.

RESULTS

Worker bees from each caste exhibited sleep signs. Results, below, include data for bees that were relatively immobile (defined above and referred to hereafter as 'immobile') and were observed either outside or inside cells. We report antennal states associated with sleep in bees as either 'antennae immobile' (i.e. antennae motionless) or 'antennae variable' (i.e. antennae motionless, slightly twitching, or exhibiting larger, usually swaying motions). Data representing these two categories allow for additional analyses of 'deep sleep' [as Kaiser (Kaiser, 1988) was tempted to call the sleep state during which antennae are immobile] *versus* total sleep exhibited outside cells, respectively. Results from nonparametric tests are consistent with parametric test results, with one possible exception, noted below.

Scan:72 h

Every 30 min we recorded the behavior of each of the 13 marked bees per worker caste that we could find, resulting in observations for approximately 6 foragers and 11 of each of the other three castes (11.1±0.1 cell cleaners, 11.3±0.1 nurse bees, 11.4±0.1 food storers, 5.8±0.2 foragers; means ± s.e.m., N=144 observations per caste, including 28 observations per day and 20 per night during each 24 h period). In all four castes, some workers exhibited a sleep state outside cells, or were immobile inside cells (see average percentages over the entire study or with respect to day or night; Table 1).

Table 1. Immobility of bees determined during the course of two studies

	Scan:72 h study (% observations)				Focal:life study (% time)			
	Outside cell, antennae immobile	Outside cell, antennae variable	Inside cell	Out + in cell	Outside cell, antennae immobile	Outside cell, antennae variable	Inside cell	Out + in cell
Cell cleaners								
Total	3.9±0.6	6.5±0.7	31.8±1.4	38.4±1.5	1.0±0.4	2.3±0.6	39.4±3.7	41.7±3.6
Day	3.1±0.6	5.4±0.9	34.2±1.9	39.7±2.0	1.1±0.5	1.9±0.6	36.5±4.9	38.5±4.7
Night	5.1±1.0	8.1±1.3	28.4±1.8	36.6±2.3	0.9±0.6	2.9±1.1	43.7±5.8	46.5±5.4
Nurse bees								
Total	4.8±0.6	9.1±0.8	19.8±1.0	28.9±1.2	2.8±0.9	4.9±1.3	17.5±2.7	22.3±2.7
Day	4.7±0.8	8.5±1.1	21.1±1.4	29.6±1.6	2.7±1.0	4.8±1.6	18.5±3.7	23.3±3.7
Night	5.0±0.8	10.0±1.2	17.9±1.6	28.0±1.9	2.9±1.6	4.9±2.0	16.0±3.7	20.9±3.8
Food storers								
Total	8.3±0.7	12.5±0.8	15.8±0.9	28.4±1.3	11.2±2.0	16.5±2.5	4.3±1.3	20.8±2.5
Day	6.8±0.9	9.8±1.0	16.0±1.2	25.8±1.6	6.6±2.1	10.6±2.7	4.1±1.6	14.6±2.9
Night	10.3±1.3	16.4±1.3	15.6±1.5	32.1±1.9	18.0±3.6	25.0±4.4	4.6±2.2	29.6±4.3
Foragers								
Total	19.2±2.0	28.5±2.4	2.5±0.6	31.0±2.5	15.1±2.0	23.6±2.7	1.6±1.1	25.2±2.8
Day	5.3±1.5	10.9±2.4	2.0±0.8	12.9±2.3	9.9±2.1	14.6±2.9	2.7±1.8	17.3±3.2
Night	38.8±2.6	53.3±2.4	3.1±0.9	56.4±2.7	22.4±3.4	36.3±4.5	0.0±0.0	36.3±4.5

Values are means ± s.e.m.
Immobile was regarded as relaxed posture with no movement except for respiratory gaster pumping or twitch of leg or tarsus, or proboscis extension.
Antennae variable = antennae immobile, slightly twitching or exhibiting larger, usually swaying motions; Scan:72 h = antennae immobile, scan sampling bees' behavior across 72 h; Focal:life = antennae immobile, focal sampling of bees' behavior at important stages of their lives – across the entire study period (Total), throughout the day (Day), or throughout the night (Night).

Sleep outside cells

Older bees slept longer and with greater 24 h periodicity outside cells than did younger bees. The percentage of observations in which relaxed, immobile bees exhibited antennal immobility did not differ between cell cleaners and nurse bees, but was greater in food storers and greatest in foragers. The same relationships held when antennal states were variable, except that nurse bees did not significantly differ from cell cleaners or from food storers (Fig. 3).

Sleep outside cells did not significantly differ between night and day for cell cleaners or for nurse bees (antennae immobile: $F_{1,568}$=1.15, =0.03, P=0.28, =0.87, with respect to worker caste; antennae variable: $F_{1,568}$=1.38, =0.50, P=0.24, =0.48, respectively), although nonparametric results may show a day–night difference with nurse bees' 'antennae variable' measure (χ^2_1=3.86, P=0.05). However, both food storers and foragers slept outside cells more during the night than during the day (antennae immobile: $F_{1,568}$=3.79, =351.66, P=0.05, <0.0001, respectively; antennae variable: $F_{1,568}$=8.87, =365.52, P=0.003, <0.0001, respectively; Figs 4 and 5). Examining sleep rhythmicity using autocorrelations, we found no evidence for diurnal or ultradian rhythms in cell cleaners or nurse bees. Food storers exhibited a perceptible 24 h periodicity when antennae were variable, and foragers displayed a strong and clear 24 h periodicity when antennae were immobile or variable (Fig. 6A,B). Sleep outside cells was impacted by worker caste, day *vs* night and the interaction of worker caste by day *vs* night (antennae immobile: $F_{3,568}$=86.91, $F_{1,568}$=120.33, $F_{3,568}$=78.76, respectively, P<0.0001 for each; antennae variable: $F_{3,568}$=107.26, $F_{1,568}$=143.74, $F_{3,568}$=77.51, respectively, P<0.0001 for each).

Immobile inside cells

The percentage of observations of bees immobile inside cells decreased across castes, with cell cleaners found most often inside cells and immobile, nurse bees and food storers found less often, and foragers found least inside cells and immobile, in contrast to the pattern of increasing worker sleep outside cells (Fig. 3). Although we observed some bees immobile inside cells more frequently than other bees, this difference existed only with respect to caste ($F_{3,568}$=133.66, P<0.0001; Fig. 3) and day *vs* night ($F_{1,568}$=4.10, P=0.04), and not with respect to interaction of caste by day *vs* night ($F_{3,568}$=2.21, P=0.09). Autocorrelations revealed no diurnal or ultradian rhythmicity of in-cell immobility for any worker caste (Fig. 6C).

When we summed the recordings of sleep outside cells with those of immobility inside cells, we found that cell cleaners slept more than their older siblings. Nurse bees and food storers slept slightly less than foragers and foragers slept less, although not statistically so, than cell cleaners. Cell cleaners and nurse bees were as immobile during the day as they were during the night ($F_{1,568}$=1.13, =0.31, P=0.29, =0.58, respectively), but food storers and foragers were immobile more often at night than during the day ($F_{1,568}$=4.48, =218.89, P=0.03, <0.0001, respectively; Figs 4 and 5). Autocorrelations revealed no rhythmicity in cell cleaners or nurse bees, the food storers' 24 h periodicity was dampened by lack of rhythmicity while immobile in a cell, and the foragers' 24 h rhythm remained significant (Fig. 6D). Sleep outside cells plus in-cell immobility were impacted by worker caste, day *vs* night, and interaction of caste by day *vs* night ($F_{3,568}$=9.70, $F_{1,568}$=58.48, $F_{3,568}$=55.44, respectively; P<0.0001 for each).

Focal:life

We continuously recorded the activity of bees at different stages of their lives: 54 as cell cleaners, 33 as nurse bees, 30 as food storers and 21 remaining foragers – two bees per hour for 15 min each bee (×2 trials), resulting in an average of 96, 15 min observation sessions of each worker caste as the bees aged. When we eliminated all data except for one observation per bee per caste, statistical significance was retained in all analyses, unless noted below.

Cell cleaners exhibited a sleep state, as did each of the subsequent age castes (see average percentages over the entire study or with

respect to day or night; Table 1). There was great variation in behaviors exhibited within each age caste. Some bees of typical nurse bee age exhibited behavior typical of cell cleaners (extended periods in empty or egg-containing cells without obvious body movement). Some bees of typical food storer and forager age did not neatly fall into their respective functional classifications. Callows introduced into hive 1 were rejected by the hive, so all data from the Focal:life study refer to hive 2.

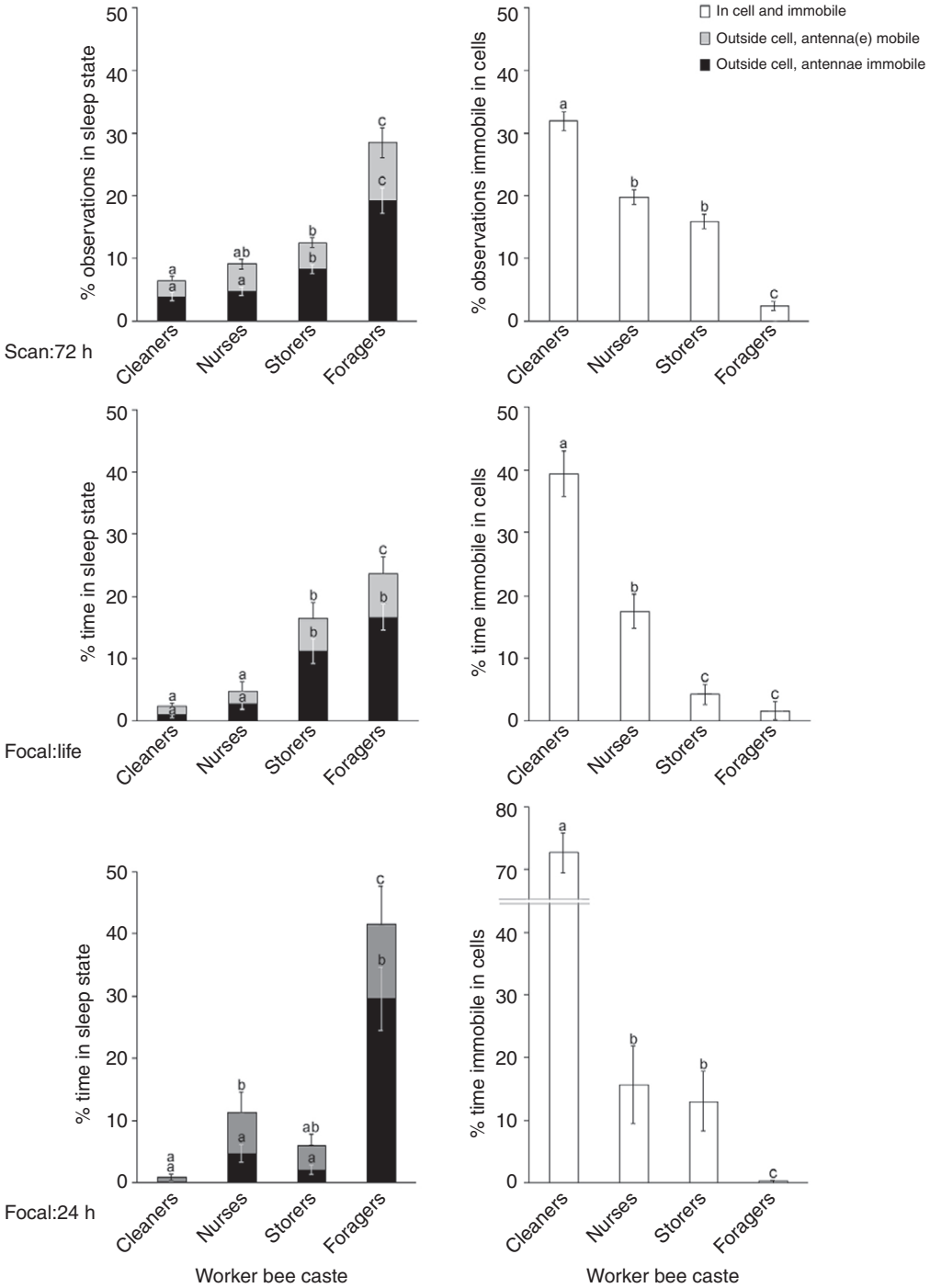

Fig. 3. Relative immobility in relaxed state with respect to worker caste. Immobile bees were observed outside comb cells with motionless antennae (black bars), with antennae twitching or exhibiting larger motions (gray bars), or bees were observed inside cells and immobile (white bars). Patterns of sleep and immobility within cells remained fairly consistent across the studies, with older bees sleeping more outside cells and younger bees spending more time immobile inside cells. Error bars indicate s.e.m. associated with black bars, black+gray bars, or white bars. Different letters indicate statistically significant differences among the castes with respect to these measures. Castes are ordered by presumed (nurse bees, food stores and foragers in Scan:72 h study) or actual (cell cleaners in Scan:72 h study, and all bees in Focal:life and Focal:24 h study) age, from left to right. (Scan:72 h) percentage observations of ~40 randomly selected marked bees; scan sampling: 3–5 s per bee every 30 min for 72 h. (Focal:life) percentage time, four bees randomly selected from same age group; focal sampling: 15 min per bee per hour for 48 h every week for four weeks of their lives. (Focal:24 h) percentage time, one bee of each worker caste; focal sampling: 30 min per hour for 24 h.

Sleep outside cells

Consistent with the Scan:72 h study, young cell cleaners and nurse bees spent less time in a sleep state with antennae immobile than after the transition from nurse bee to food storer, but time spent in this sleep state did not increase after food storers became foragers. In the antennae variable group, the same pattern of age-dependent sleep increase occurred, except that foragers spent more time in a sleep state than any younger age caste ($F_{3,379}$=36.91, P<0.0001; Fig. 3). This statistical increase from food storer to forager was not retained after we eliminated all data except for one observation per bee per caste.

Sleep outside cells did not significantly differ between night and day for cell cleaners or for nurse bees (antennae immobile: $F_{1,379}$=0.00, =0.01, P=0.95, =0.94, respectively; antennae variable: $F_{1,379}$=0.06, =0.00, P=0.80, =0.98, respectively). As food storers and foragers, however, bees slept outside cells more during the night than during day (antennae immobile: $F_{1,379}$=15.18, =18.56, P=0.0001, =0.00002, respectively; antennae variable: $F_{1,379}$=14.37, =32.81, P<0.0001, =0.0002, respectively; Figs 4 and 5). Sleep outside cells was impacted by worker caste, day *vs* night and the interaction of worker caste by day *vs* night (antennae immobile: $F_{3,379}$=25.62, $F_{1,379}$=17.03, $F_{3,379}$=5.65, P< 0.0001, <0.0001, =0.0009, respectively; antennae variable: $F_{3,379}$=33.14, $F_{1,379}$=24.14, $F_{3,379}$=7.80, P< 0.0001 for each, respectively). When we eliminated all data except for one observation per bee per caste, one difference was lost: foragers did not show a night–day difference.

Immobile inside cells

The percentage of time spent in cells (immobile or not) decreased when cell cleaners became nurse bees and decreased again when nurse bees became food storers (45.7±3.9%, 29.5±3.2%, 8.7±1.7%, respectively). There was no significant change in time spent in cells when food storers became foragers (4.0±1.7%). Time spent immobile inside cells followed the same caste-dependent pattern ($F_{3,379}$=49.40, P<0.0001; Fig. 3). Time immobile inside cells was not impacted by day *vs* night, or by the interaction of caste by day *vs* night ($F_{1,379}$=0.06, $F_{3,379}$=0.84, P=0.81, =0.47, respectively). When we eliminated all data except for one observation per bee per caste, time spent immobile inside cells decreased with age, but the statistical difference between nurse bees and food storers, and food storers and foragers was not retained.

Nearly all results pertaining to the combined measure of sleep outside cells and immobility inside cells are consistent with the Scan:72 h study. Bees spent more time immobile (outside+inside cells) as cell cleaners than during any subsequent stage of their adult lives (Fig. 3; combining outside+inside cell percentages). Cell cleaners and nurse bees spent as much time immobile (outside+inside cells) during the day as they did during the night ($F_{1,379}$=1.92, =0.17 and P=0.17, =0.68, respectively). As food storers and foragers they spent more time immobile at night than during the day ($F_{1,379}$=6.50, =10.56, P=0.01, =0.001, respectively; Figs 4 and 5). Worker sleep or in-cell immobility were impacted by caste, day *vs* night, and interaction of caste by day *vs* night ($F_{3,379}$=10.94, $F_{1,379}$=11.51, $F_{3,379}$=2.59, P<0.0001, =0.0008, =0.05, respectively). Differences in day *vs* night were not retained after we eliminated all data except for one observation per bee per caste.

Sleep bouts

Uninterrupted sleep bouts were not only longer in the older bees (longer in food storers and foragers than in cell cleaners and nurse bees), but lasted longer during the night than during the day (Fig. 7). Unbroken bouts of immobility inside cells decreased as bees aged, both when cell cleaners became nurse bees and again when nurse bees became food storers (Fig. 7), although when we eliminated all data except for one observation per bee per caste the only statistical difference retained was between cell cleaners and older castes. Maximum durations of unbroken sleep bouts while outside cells were 89, 180, 330, and 333 s for cell cleaners, nurse bees, food storers and foragers, respectively. Of these periods, 89, 162, 330 and 303 s were maximum unbroken periods with antennae immobile. Immobility inside cells sometimes exceeded entire 900 sec census periods for cell cleaners and nurse bees and lasted at most 463 and 653 s for food storers and foragers, respectively. Unbroken bouts of sleep spent outside cells were impacted by caste, day *vs* night,

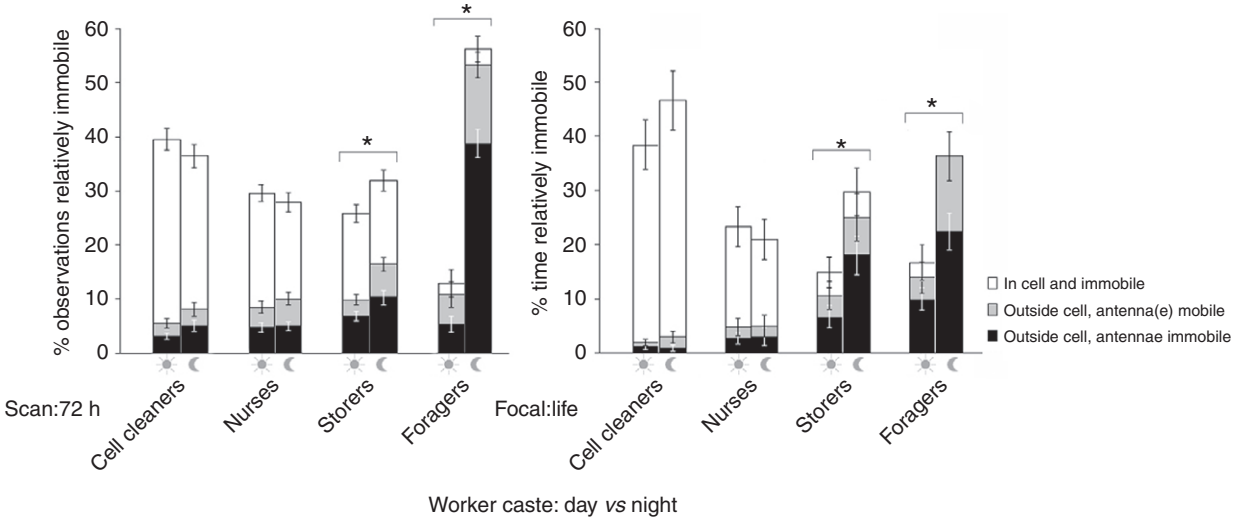

Fig. 4. Relative immobility in relaxed state during the day (sun) *versus* during the night (moon) with respect to worker caste. Food storers and foragers sleeping more, by any cumulative measure, during the night than during the day. Error bars indicate s.e.m. associated with black bars, black+gray bars, or black+gray+white bars. The asterisk signifies a statistically significant difference between daytime and nighttime measurements with respect to these measures. All worker castes spent the same amount of time immobile inside cells during the night as during the day, so measure of 'in cell and immobile' did not alter night–day differences for any worker caste. (Scan:72 h), % observations. (Focal:life), % time.

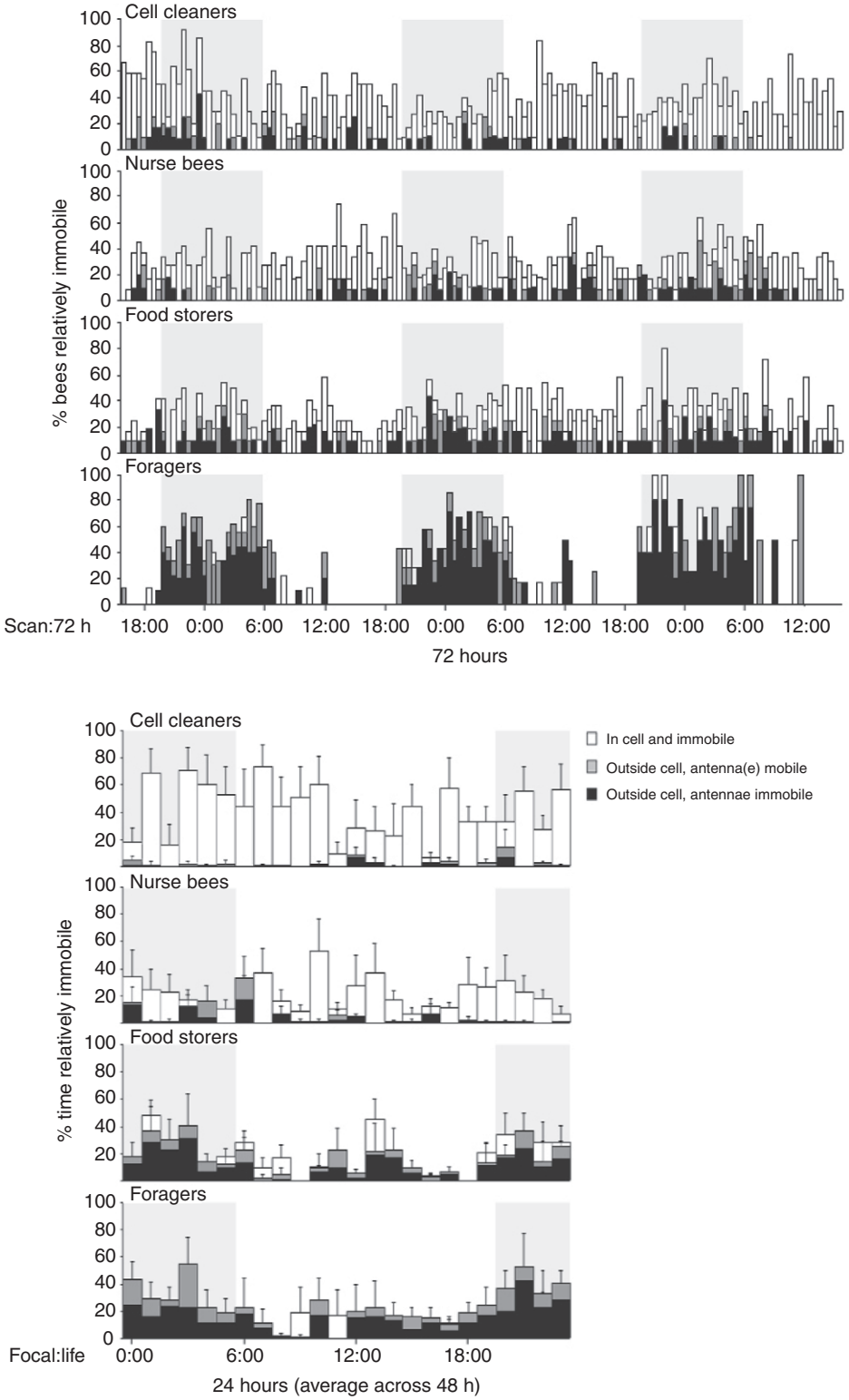

Fig. 5. Timing of sleep outside cells or immobility inside cells with respect to worker caste. Worker bees were observed in an immobile, relaxed state outside comb cells with motionless antennae (black bars), with antennae slightly twitching or antennae exhibiting larger motions (gray bars), or inside cells (white bars). Shaded backgrounds indicate nighttime. Castes are ordered by behavior/function (Scan:72 h study) or age (Focal:life study). (Top; Scan:72 h) Percentage of bees in sleep state outside cells or immobile inside cells. Scan sampling: each bee recorded (if found) every 30 min for 72 h. (Bottom; Focal:life) percentage of time bees (N=4 per hour) spent in sleep state outside cells or immobile inside cells. Error bars reflect s.e.m. margins associated with black+gray bars, or white bars.

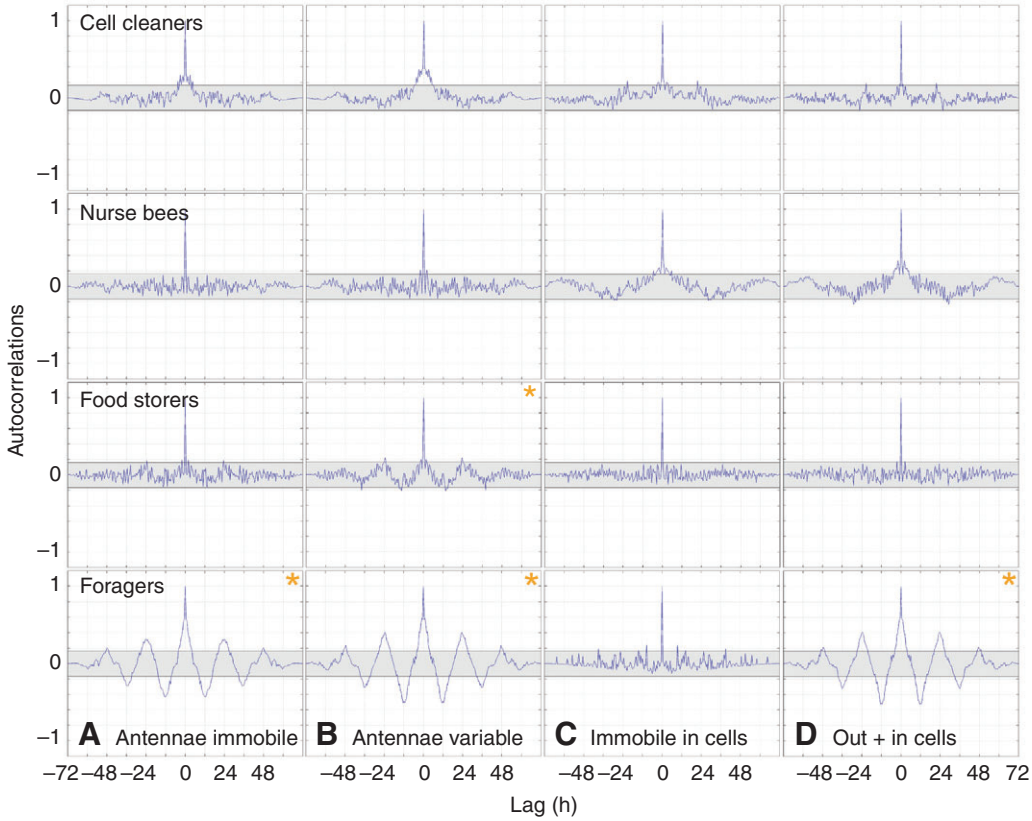

Fig. 6. Autocorrelations conducted on Scan:72 h data, assessing rhythmicity of worker castes in relaxed posture exhibiting relative immobility when (A) antennae were immobile, (B) antennae were variable, (C) immobile inside cells, and (D) the sum of A, B and C. Food storers exhibited a 24 h rhythm when antennae were variable, and foragers exhibited strongly significant 24 h rhythmicity when antennae were immobile, variable, and when all measures were summed. There was no evidence for ultradian or circadian rhythms (i.e. large oscillations were absent) for cell cleaners, nurse bees or any in-cell immobility. An asterisk indicates periodicity and shaded region represents the 95% confidence intervals. A peak at the center of each graph indicates zero lag (amount of shift) and therefore perfect correlation.

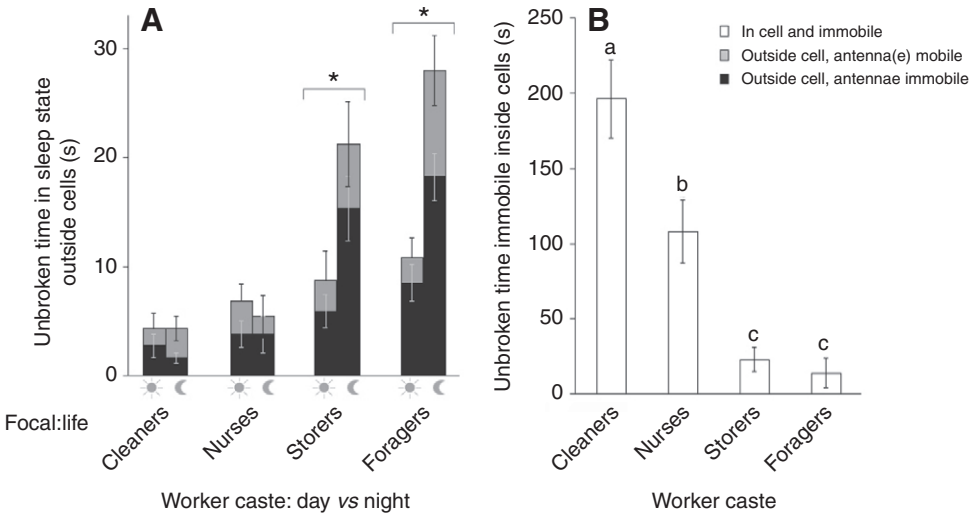

Fig. 7. Uninterrupted bouts of relative immobility with respect to worker caste. (A) Outside cells: food storers and foragers exhibited longer unbroken sleep bouts during the night (moon) than during the day (sun), as measured by antennal immobility (black bars) or in combination with antennae slightly twitching or exhibiting larger motions (gray bars). (B) Inside cells (white bars): unbroken bouts of relative immobility decreased with age. No difference existed between night and day, so data were collapsed for each caste. Worker bees were observed for 48 h during each of four stages of their adult lives (cell cleaners to foragers, respectively). An asterisk indicates a significant difference between daytime and nighttime measurements of black and black+gray bars and different letters indicate statistically significant differences among white bars. Error bars indicate s.e.m. associated with black bars, black+gray bars, or white bars.

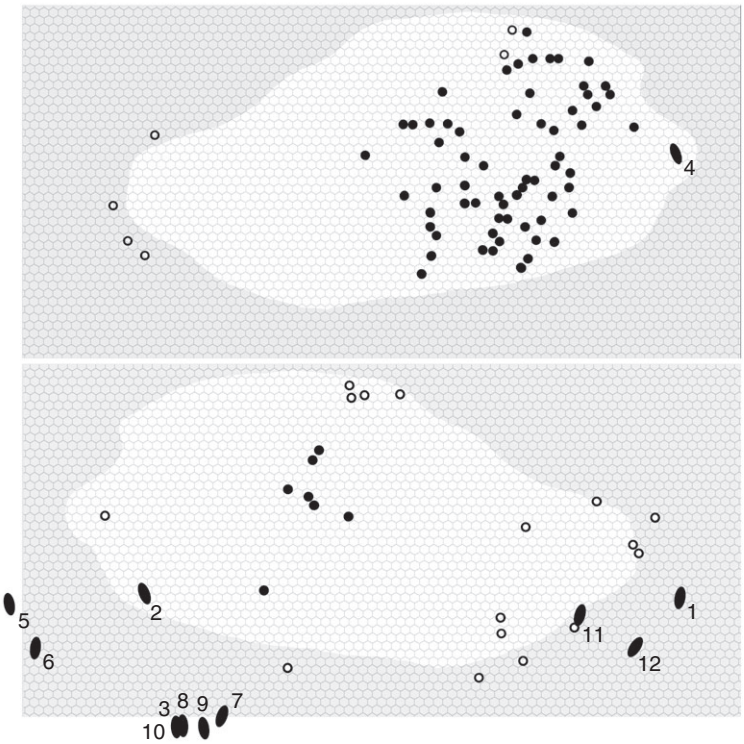

Fig. 8. Activity of three bees, mapped on opposite sides of the bottom frame of the observation hive, indicating that the forager slept on the periphery of the hive, the cell cleaner visited cells within the brood comb area, and the food storer visited cells on the edge of the brood comb. White space roughly outlines brood comb on 13 August. Filled circles, cell cleaner's cell visits; open circles, food storer's cell visits; black ovals, 12 nighttime sleep sites of the forager, numbered chronologically. The three bees were observed on separate days continuously for 30 min per hour for 24 h.

and the interaction of caste by day *vs* night (antennae immobile: $F_{3,379}$=20.54, $F_{1,379}$=14.74, $F_{3,379}$=6.05, P<0.0001, =0.0001, =0.0005, respectively; antennae variable: $F_{3,379}$=19.66, $F_{1,379}$=19.24, $F_{3,379}$=8.11, P<0.0001 for each).

Focal:24h

The cell cleaner spent 74% of her 24 h (30 min observation per hour) in at least 70 different cells, including 54 egg, one larval, six empty and nine unknown, all positioned within the centrally located brood comb (Fig. 8). The nurse bee spent almost all of her time in the brood area and tended the queen, but she also spent time in three egg cells and a pollen cell. The food storer spent time (range: 16–1520 s) in at least 22 different cells (ten pollen, three larva, seven egg, two empty; Fig. 8). The forager spent no time in cells and was either immobile, engaging in trophallaxis, locomoting, or foraging (every hour from 07:00–11:00 h). The forager exhibited no sleep site fidelity, although she spent all of her sleep state periods close to the periphery of the brood comb (Fig. 8).

Sleep outside cells

The forager spent more time outside cells in a sleep state than the cell cleaner, nurse bee, or food storer (antennae immobile: $F_{3,92}$=25.18, P<0.0001; antennae variable: $F_{3,92}$=26.61, P<0.0001; Fig. 3).

Immobile inside cells

The cell cleaner spent more time immobile inside cells than her older sisters; the forager spent no time in cells. As with the Scan:72h and Focal:life studies, the cell cleaner spent more time immobile, in or out of cells, than older bees, because of her extended immobile periods spent inside cells ($F_{3,92}$= 20.73, P<0.0001; Fig. 3).

Sleep bouts

Whereas the cell cleaner spent no time outside cells in a sleep state, the nurse bee and food storer spent an average of 5 s with antennae immobile and 9–13 s with antennae variable. The forager spent the most time outside cells in a sleep state: 40±13 s (antennae immobile) and 49±12 s (antennae variable). The reverse pattern occurred with respect to immobility inside cells: the cell cleaner spent more time immobile than the nurse bee and food storer (361±56, 123±65, 119±43 s, respectively) and the forager spent no time in cells.

DISCUSSION

Our investigation of sleep in honey bees revealed differences in sleep among the four worker castes, expanding on previous work distinguishing sleep duration, constitution and periodicity in honey bees.

Duration and constitution

Patterns of sleep and immobility were consistent across the studies, with bees sleeping more outside cells when older, and spending more time immobile inside cells when younger. This increase in sleep outside cells with respect to age/caste held true for total antennal immobility, a state correlated with high arousal threshold and speculated to be the deepest state of sleep (Kaiser, 1988). Cell cleaners and nurse bees exhibited more sleep and more deep sleep outside cells when they became food storers and, in the case of variable antennae, again as food storers became foragers (Fig. 3). As younger bees aged and changed tasks, they also experienced longer unbroken bouts of sleep outside cells, increasing as nurse bees became food storers, and again as food storers became foragers, but experienced shorter bouts of immobility inside cells (Fig. 7). Our caste-dependent sleep and immobility data are consistent with some, but not all of Moore et al.'s findings (Moore et al., 1998).

Periodicity

We detected no diurnal or ultradian sleep cycles in cell cleaners or nurse bees, but did detect a 24 h sleep:wake periodicity in food storers and, as expected, a strong 24 h sleep:wake periodicity in

foragers (Fig. 6). Food storers and especially foragers spent more time asleep, and had longer unbroken bouts of sleep, during the night than during the day (Figs 4 and 7, respectively). We found no evidence of rhythmicity of in-cell immobility for any worker caste (Fig. 6). See below for comparisons with other studies.

For all of our work we used a proxy for the set of behavioral characters deemed diagnostic of sleep. Because it was impossible to examine all sleep signs simultaneously, we recorded relative immobility (defined above) when bees were inside cells or outside cells. Bees may perform wakeful actions that are undetectable under normal conditions, like voluntary head and mouthpart movements inside cells, or wing muscle contractions for heat production either inside or outside cells (Esch, 1960). Fortunately, bees performing certain wakeful actions can be distinguished from resting bees by the temporal periodicity of their gaster pumping movements (discontinuous in resting bees; continuous in 'heating' bees). Cell cleaners, for instance, have been shown to spend almost their entire time discontinuously ventilating (and staying relatively immobile) while inside cells (Sauer et al., 1998) (B.A.K., unpublished). Heating bees can also be distinguished from resting bees by the heat generated from their alitrunks (Kleinhenz et al., 2003) or by their posture (Bujok et al., 2002). Discontinuous ventilation covaries with antennal immobility (Sauer et al., 2003), so respiratory rate could potentially serve as a proxy, or even exclusive indicator of sleep in bees inside cells.

Our categories of sleep differ from Moore et al.'s (Moore et al., 1998) categories of presumed inactivity and differ somewhat from Kaiser's (Kaiser, 1988) categories of honey bee sleep. Moore et al. recorded observations of workers either 'standing' (i.e. motionless, not in cell), or 'motionless in cell' (i.e. bee remains in cell for longer than 3 min). These categories differ from our categories in that Moore et al. did not distinguish between non-rest immobile behavior and relaxed immobility and they did not distinguish between antennal immobility and mobile states of antennae. Also, Moore et al. measured in-cell immobility by duration (>3 min) inside cell, not solely by lack of movement within cell. Kaiser's classification of 'immobile' antennae included 'sporadic, minute movements'. We have divided this into 'antennae immobile' and 'antenna(e) twitching' (see Movies 2 and 3 in supplementary material), although our distinction was based on close observation and not via automated infrared detection in tethered subjects. Kaiser also noted separate categories of smaller and larger antennal movements, which we speculate falls under our designation of 'antennae variable'. Our conclusions are probably unaffected by this potential discrepancy, although future work should be careful to define precisely each behavioral category recorded. Clarity on this will facilitate replication of experiments and interpretation of results, which could be especially important should new insights reveal an important functional difference between the two antennal states.

Our research does not definitively address the importance of age *versus* task in determining sleep behavior. The most informative experiment to distinguish the effects of age and task occurred when Bloch and Robinson (Bloch and Robinson, 2001) induced a reversion of 'rest' behavior in foragers. They found that by inducing foragers to re-engage in nurse bee behavior, they produced bees that no longer exhibited circadian 'rest', although this work did not report on possible changes in duration of sleep or on behavior within cells. Although we can be certain of the relatively young age of cell cleaners in all of our studies, and of the age of all bees in the Focal:life and Focal:24h studies, we cannot be certain of the age of the nurse bees, food storers and foragers in the Scan:72h study, because they were chosen based on their behavior, not their age. Most of the nurse bee-, food storer- and forager-aged bees within the Focal:life study fell within the behavioral categories defined by Sakagami (Sakagami, 1953) and Seeley (Seeley, 1982).

Until now, no study has explicitly examined the sleep:wake patterns of recently eclosed bees in the context of the colony. Lindauer's (Lindauer, 1952) observations of cell cleaners confounded other behaviors with sleep, and other studies have measured *activity* rhythms of newly eclosed cell cleaners maintained in isolation. Spangler (Spangler, 1972), Sauer et al. (Sauer et al., 1998; Sauer et al., 1999), and Moore et al. (Moore et al., unpublished) measured the locomotor activity of recently eclosed worker bees kept in constant darkness by using either vibration sensors (Spangler, 1972) or infrared sensors. Sauer et al. (Sauer et al., 1998; Sauer et al., 1999) placed each bee within a cuvette, provided her with a small piece of comb containing food, and reported that these bees, like those in our study, exhibited behavioral sleep signs and gradually gained a 24 h rest:activity rhythm as they grew older. Contrary to our autocorrelation results, however, Sauer et al.'s analyses identified and quantified an ultradian rhythm of rest:activity within the first 24 h, and subsequent days, following eclosion.

Having examined bees older than cell cleaners ($\geqslant$4 days after eclosion), Moore et al. (Moore et al., 1998) reported more in-cell immobility during the night than during the day – across age bins and in both genotypes of honey bee studied. They also reported more 'standing' during the night than during the day, albeit only in one of the two genotypes. These data, suggesting greater rest during the night than during the day in worker bees, are consistent with Lindauer's (Lindauer, 1952) observations and our autocorrelation data for older worker bees, but not consistent with our data for nurse bees.

One of Sauer et al.'s (Sauer et al., 1998) bees, observed for 21 days, developed a 12 h rest:activity cycle during food storer age and developed a circadian rhythm within 15–21 days after eclosion. If Sauer et al.'s measurements of rest:activity reflect sleep:wake cycles, our data do not support a 12 h sleep:wake cycle in food storer aged bees; instead our food storer data fall along a sinusoidal path that suggests 24 h periodicity of sleep (Fig. 6B). Although oscillations were not large in these food storer data, Levine et al. (Levine et al., 2002) argue that autocorrelation data that follow a clear pattern but may not achieve statistical significance due to small sample size (e.g.<180), should be interpreted as circadian/periodic.

A major component of our study focused on duration and (lack of) periodicity inside comb cells. Little is known about what honey bees do inside cells. Cell cleaners enter for periods that can last over an hour and a half, often engaging in no discernible activity (Sakagami, 1953; Sauer et al., 1998). Kolmes (Kolmes, 1984) referred to periods lasting more than 3 min inside a cell simply as 'in cell'. Lindauer (Lindauer, 1952), by examining young bees entering cells abutted against glass in a specially designed observation hive, determined that callows occasionally clean cells. This behavior typically vanishes after the third day of adulthood, sometimes accounting for <1% of observations (Moore, 1998). In spite of some ambiguity, callows are frequently referred to as 'cell cleaners' throughout the literature ['Zellenputzen' by Lindauer (Lindauer, 1952)] (Sakagami, 1953; Seeley, 1982; Seeley, 1991).

Although callows appear to perform some cell cleaning, few studies have attempted to distinguish or quantify what they may spend the majority of their time doing within cells: resting (or sleeping). Lindauer (Lindauer, 1952) recorded 'idlers' spending 30 min or longer inside an empty cell or egg-containing cell, but

his extended observations of two bees did not distinguish rest from active grooming and did not quantify rest inside cells. Kleinhenz et al. (Kleinhenz et al., 2003) distinguished resting bees from heating bees inside cells, but did not report durations or timing of in-cell rest, or which bees engaged in this behavior. Kaiser (Kaiser, 1988) was well aware that bees appeared to rest while in cells, but found that isolated bees supplied with a small piece of comb did not always rest within the cells, so he chose to study only bees outside cells to increase the visibility and continuity of measurements of his subjects. Sauer et al. (Sauer et al., 1998) reported that isolated bees were frequently observed in cells when young and that between brief alterations of body positions bees in cells were always observed discontinuously ventilating. Moore et al. (Moore et al., 1998) quantified 'motionless in cell', beginning on day 4 of adulthood and discovered in-cell immobility to be consistently rhythmic, showing greater exhibition of this 'presumed inactive' behavior during the night than during the day. Our data do not demonstrate this day:night difference with respect to in-cell immobility for any caste (Figs 4 and 6). Moore et al.'s data also show no consistent age-dependent differences with regard to duration spent motionless in cells, in contrast to our data, which show a decrease with respect to age (Fig. 3). As a result of this discrepancy, Moore et al.'s data show that forager-aged bees remained motionless in cells considerably more than we found to be the case for our foragers. Moore et al. (Moore et al., 1998), by recording rhythmicity and duration of immobility in cells, Sauer et al. (Sauer et al., 1998), by suggesting that all in-cell time was spent discontinuously ventilating, and Kleinhenz et al. (Kleinhenz et al., 2003), by distinguishing measures of body temperature and respiratory movement between resting and heating bees, may have produced the only work (until the present study), following Lindauer's (Lindauer, 1952) observations, specifically addressing potential sleep occurring inside cells. If relative immobility within cells is an indicator of sleep, then our study demonstrates that the youngest adult bees (cell cleaners) exhibit the most sleep (Focal:life and Focal:24h studies), or more sleep than nurse bees and food storers and at least as much as foragers (Scan:72h study). This in-cell measure of potential sleep could compensate for what would otherwise indicate an exceptional increase of sleep in an aging animal (when sleep outside cells, and not immobility inside cells, is considered).

It is possible that cell cleaners are less a functional caste and more a transitional state between subimago and mature adulthood, during which bees primarily sleep. And why sleep within cells? Unlike foragers, which may sleep more frequently on the periphery of the comb [see Kaiser (Kaiser, 1988) for his observations of unmarked bees presumed to be foragers, and Fig. 8 for our data on sleep sites in one forager], cell cleaners spend nearly all of their time within the bustling brood comb [see p. 34 of Seeley (Seeley, 1985)] (Fig. 8 for our 24 h examination of cell visits by a cell cleaner). Slipping into brood cells to sleep may help tired, young bees avoid disturbance by active colony mates and it may offer protection and warmth that could conceivably contribute to regenerative processes or cognitive development. Kaiser et al. and Schmolz et al. (Kaiser et al., 2002; Schmolz et al., 2002) independently discovered that the voluntary sleep sites of foragers along an artificial thermal gradient [and within an observation hive (Schmolz, 2002)] exceeded ambient temperature and concluded that reasons other than energy conservation may be linked to this preference. Schmolz et al. speculate that the reduced thermoregulatory behavior of sleeping bees could serve as a compromise between energy conservation and regenerative processes.

Flexibility in sleep behavior may be one more hallmark of the adaptive flexibility exhibited by honey bees. Within-colony variation and individual plasticity with respect to sleep:wake schedules could enhance a colony's ability to effectively exploit changing resources (Klein and Seeley, 2007) and respond to brood fluctuations, parasite invasions or predator attacks. Nurse bees, for example, might respond to the colony's arrhythmic demand for hygiene and brood care by exhibiting a lack of rhythmic sleep, whereas food storers might sleep in response to a nocturnal lull in the foragers' food collecting, but then awaken when incoming food needs to be unloaded and processed.

Kaiser (Kaiser, 1988) has compared sleep in bees to sleep in mammals, referring not only to the behavioral characters defining sleep, but also to differences with respect to timing of 'deep' sleep, specifically in humans. Sauer et al. (Sauer et al., 1998) reported that honey bee workers exhibited more circadian organization to the timing of sleep as they aged – a phenomenon shared by humans, but not by *D. melanogaster* (Koh et al., 2006). Later, Sauer et al. (Sauer et al., 2003) reported another sleep measure shared with humans: foragers exhibited less sleep as they age. They partly attributed the reduction in sleep as foragers age to a possible influence of the stress of experimental isolation. In spite of the lack of control and precision, there is value in examining sleep behavior within the context of a society. Future investigations of sleep in its natural setting might further reveal the similarities and differences between honey bee sleep and human sleep. For instance, although little is known about prenatal human sleep, sleep in immature stages of insects remains a complete mystery. Holometabolous insect development features changes through immature stages (egg, larval stadia, pupa) that may serve as important periods during which a sleep-like state could play a critical role in proper development and function of the organism, as has been proposed for prenatal humans.

Understanding the frequency and degree to which individuals of different castes within an insect society sleep is the starting point from which one can test and understand the nature of activity patterns, sleep, and sleep's impact on the behavior and ecology of societies, as well as societally based purposes of sleep.

We dedicate this paper to Dr Walter Kaiser for his pioneering work on sleep research with honey bees. We thank the Department of Neurobiology and Behavior at Cornell University for the use of the Liddell Field Station, including living space for round-the-clock bee observations. B.A.K. would also like to thank Walter Kaiser for bee sleep discussions, Shanna Smith for statistics consultation, Daniel Bushey for supplying circadian analysis tools, Sasha Mikheyev, Jack Grinband, Eben Gehring and Juan Carlos Santos for helpful discussions and, of course, the honey bees. Darrell Moore, Ulrich Mueller and members of his lab, two anonymous reviewers, and Nim Lee shared comments on earlier drafts of this manuscript. The University of Texas at Austin's Dorothea Bennett Memorial Graduate Research Fellowship funded transportation between Austin and Ithaca.

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