

Chapter 1.3

Circadian Rhythms and Sleep in Honey Bees

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Abstract The circadian clock of the honey bee is involved in complex behaviors and is socially regulated. Initial molecular characterization suggests that in many ways the clock of the bee is more similar to mammals than to *Drosophila*. Foragers rely on the circadian clock to anticipate day–night fluctuations in their environment, time visits to flowers, and for time compensation when referring to the sun in sun-compass orientation and dance language communication. Both workers and queens show plasticity in circadian rhythms. In workers, circadian rhythms are influenced by task specialization and regulated by direct contact with the brood; nurse bees tend the brood around the clock with no circadian rhythms in behavior or clock gene expression. An important function of the circadian clock is the regulation of sleep. Bees show a clear sleep state with a characteristic posture, reduced muscle tonus, and elevated response threshold. Honey bee sleep is a dynamic process with common transitions between stages of deep and light sleep. The sleep stages of workers active around-the-clock are overall similar to foragers. Sleep deprivation leads to an increase in the expression of sleep characteristics the following day, and may interfere with some learning paradigms. This review shows that the honey bee is an excellent model with which to study circadian rhythms and sleep in an ecologically and socially relevant context. Future research needs to deepen our understanding of these fascinating behaviors, reveal their neuronal and molecular bases, and explore their interactions with other physiological processes.

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Abbreviations (Excluding Gene and Protein Names)

| | |
|------|--------------------|
| FS | First sleep stage |
| MB | Mushroom body |
| mRNA | messenger RNA |
| OL | Optic lobe |
| SS | Second sleep stage |
| TS | Third sleep stage |

1.3.1 Circadian Rhythms

1.3.1.1 What Are Circadian Rhythms?

Circadian rhythms are defined as biological rhythms that meet the following three criteria: (1) they persist, or “*free-run*”, with a period of about 24 h in the absence of external time cues, (2) they are reset, or *entrained*, by environmental cues, in particular light and temperature, and (3) they have a stable period length in a wide range of physiologically relevant temperatures. This phenomenon, commonly termed ‘*temperature compensation*’, is thought to require specific mechanisms because most biological reactions accelerate with rising temperature. The circadian clock influences many physiological and behavioral processes. These include activity, sleep-wake cycles, feeding, mating, oviposition, egg hatching, and pupal eclosion. The circadian clock is also involved in measuring day length, and influences photoperiodism and annual rhythms such as diapause and seasonal reproduction [12].

The circadian system is commonly described as having three functional components. The *core* of the clock is composed of *pacemakers*, cell autonomous rhythm generators that cycle approximately, but not exactly with a 24-h period. The central pacemaker is entrained by *input pathways* in which environmental signals are detected, converted to sensory information, and transmitted to the central pacemakers. *Output pathways* carry temporal signals away from pacemaker cells to various biochemical, physiological, and behavioral processes [2, 12].

The molecular bases of rhythm generation in organisms as diverse as fungi, plants, fruit flies and mammals consists of interlocked autoregulatory transcriptional/translational feedback loops with positive and negative elements [12]. The pacemaker cells are interconnected in a circadian network that couples their activities and orchestrates normal rhythms in physiology and behavior [2, 12].

The molecular clockwork of the fruit fly *Drosophila melanogaster* has been well characterized and provides a model for studies on animals, and insects in particular. The positive elements *Clock (Clk)* and *Cycle (Cyc)* activate the transcription of the negative elements, the transcription factors *Period (Per)* and *Timeless (Tim1)*. *Per* and *Tim1* are translated into proteins that enter the nucleus where they interfere with the transcriptional activity of the CLK: CYC complex, and by that shut down their

own expression. *Par Domain Protein 1 (Pdp1)*, *Vrille (Vri)*, and *Clockwork Orange (Cwo)* are thought to act together with *Clk* in an interlocked feedback loop that is thought to stabilize the *Per/Tim1* loop. Several kinases including *Double-time (Dbt)*, *Shaggy (Sgg)*, *Casein Kinase II (CKII)* and *Protein Phosphatase 2A (PP2A)* fine tune this cell-autonomous rhythm generation machinery [2, 12]. *Drosophila*-type *Cryptochrome (Cry-d)*, also known as *insect Cry1* has a photic input function. Although the genes and the organization principles of the molecular clockwork are similar in *Drosophila* and mammals, there are some important differences. For example, mammals do not have orthologs to *Tim1* and *Cry-d*, but have three paralogs for *Per*. They also have two paralogs for *Cry* (mammalian-type *Cry*) that act together with the *Per* genes in the negative loop of the clock.

1.3.1.2 Circadian Rhythms in Honey Bees

Circadian rhythms in the honey bee have been recently reviewed in Bloch [3] and [4] and are therefore only briefly discussed below. The first behavior with a rhythm of about a day described for bees was the flying of foragers outside the hive at a specific time of day [46]. The observation that foragers can learn to associate a food reward with a specific time of day led to the discovery that the circadian clock is involved in **time memory** (“Zeitgedächtnis”). Bees have excellent time memory and can learn to arrive at a specified location at any time of the day; they can learn as many as 9 time points with intervals of only 45 min between food availability (reviews: [3, 27]). Foragers also rely on their circadian clock to compensate for the sun’s movement with time (**time-compensated sun-compass orientation**), since they orient themselves by maintaining a fixed angle to the sun, and the sun moves during the day. Foragers staying for long periods inside the hive use the clock to correct their waggle-dance in accordance with the shift in the sun’s azimuth [47]. **Locomotor activity**, the best studied behavioral circadian rhythm in animals, has been well-characterized in honey bees (reviews: [3, 27]).

1.3.1.3 The Molecular and Neuronal Organization of the Honey Bee Circadian Clock

The honey bee genome does not encode orthologs to *Cry-d* and *Tim1* genes, but does have orthologs to the mammalian-type paralog *Cry-m* (also known as insect *Cry2*) [35]. The CRY-m proteins of bees and other insects, like mammalian CRY proteins, are effective transcriptional repressors and are not sensitive to light. Thus, *amCry-m* is not likely to fill the photic input function of *Drosophila Cry* [50]. The absence of orthologs to *Cry-d* and *Tim1* and the evidence that *Cry-m* is not sensitive to light suggest that honey bees use a novel light input pathway. The honey bee

genome also encodes a single ortholog for the clock genes *Per*, *Cyc*, *Clk*, *Cwo*, and *Tim2*. Furthermore, there are highly conserved orthologs to *Vri* and *Pdp1*, but no true orthologs to the orphan nuclear receptors REV-ERB (α and β) and ROR (α , β , γ) that are thought to orchestrate the expression of BMAL1 (the vertebrate ortholog of *Cycle*) in the mammalian clock [35]. These findings suggest that amVRI and amPDP1 are involved in an interlocked loop regulating *amCyc* expression, reminiscent of their function in the *Drosophila* clockwork (in which they regulate *Clk* expression). This hypothesis, however, has not yet been explicitly tested.

In foragers, and other bees with strong circadian rhythms, brain mRNA levels of both *Cry-m* and *Per* oscillate with strong amplitude and with a similar phase under both light–dark and constant darkness illumination regimes. In contrast to *Drosophila*, the predicted honey bee CYC protein contains a transactivation domain and its brain transcript levels oscillate virtually in anti-phase to *Per*, as in the mouse [35, 42]. Based on the known organization principles of the molecular clockwork, and studies on clock genes in the honey bee, a working model for the honey bee circadian clock can be proposed (Fig. 1.3.1). In this model *amPer* and *amCry-m* act together as the negative elements of the interlocked feedback loop and *amCyc* is the oscillating factor in the positive limb that probably also includes *Vri* and *Pdp1*.

The anatomical organization of the circadian clock has not been described in detail for the honey bee or for any other bee. The current picture of the anatomical organization of the brain clock is based largely on immunocytochemical studies with antibodies against PER and the neuropeptide Pigment Dispersing Factor (PDF) [7, 49]. Both the PER-ir and PDF-ir clusters are located in brain areas that are implicated in the regulation of circadian rhythms in *Drosophila* and other insects. The most consistent PER immunoreactivity (PER-ir) was detected in the cytoplasm of about eight large cells in the area between the calyces and the alpha and beta lobes of the mushroom bodies. Additional neurons in the optic lobes (OLs) and other parts of the brain showed nuclear staining.

1.3.1.4 Plasticity in Circadian Rhythms and Its Social Regulation

By contrast to most insects, newly-emerged honey bees typically have no circadian rhythms in locomotor activity or metabolism (Fig. 1.3.2a; reviews: [3, 27]). The ontogeny of circadian rhythms is endogenous because it occurs under constant conditions and rhythms free-run with a period of about, but not exactly 24 h. The development of overt circadian rhythms is associated with age-related changes in brain *Per* expression (reviews: [3, 4]).

In colonies foraging in the field the expression of behavioral rhythms is associated with worker age and task specialization. Young workers typically care for the brood around-the-clock inside the constantly dark and homeostatically regulated hive and sleep in irregular intervals [28, 42, 13, 20] (Fig. 1.3.2b). Foragers have strong circadian rhythms with a consolidated period of sleep during the night [5, 18–20, 28, 42]. Honey bee larvae are frequently attended by nurse bees. Around-the-clock activity

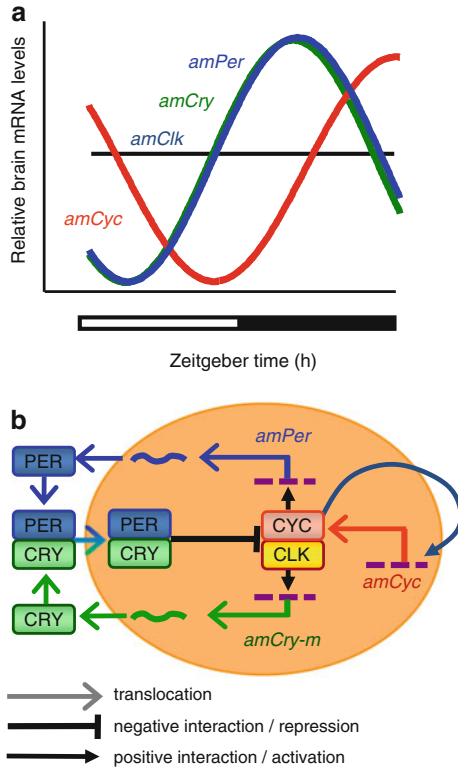


Fig. 1.3.1 A model for the honey bee molecular clockwork **(a)** Schematic representation of the oscillations of clock genes in the honey bee brain. The phase of mRNA cycling is shown for *Period* (*amPer*), *Cryptochrome-m* (*amCry*) and *Cycle* (*amCyc*). The phase of *amCyc* transcript is almost in anti-phase to that of *amPer* and *amCry*. The plots were generated by fitting a cosine model with about a 24 h cycle to brain mRNA levels measured in [35]. A straight line is depicted for *Clock* (*amClk*) that appears to have similar transcript levels throughout the day. A model is not shown for *Timeout* (*amTim2*) for which the pattern of mRNA variation over time was not consistent across experiments. Relative amplitudes for the various genes are not to scale. The bar at the *bottom* of the plot shows the illumination regime. Filled box – night or subjective night; *open box* – day or subjective day. **(b)** A schematic working model of the molecular clockwork in the honey bee brain. Gene name abbreviations in capital letters and italic lower case letters refer to proteins and DNA locus, respectively. The mRNA and protein for each gene is illustrated by similarly colored wavy lines and geometric shapes, respectively. The orange oval shape illustrates the nucleus, purple dashed lines depict DNA sequences, arrows with open wings depict translocation, arrows with closed wings depict positive interactions/activation, lines with a T end depict negative interactions/repression. The putative positive loop for *Cyc* is shown with no details (Reproduced by permission from [4])

may therefore enable nurses to provide better care for the brood, whereas foraging is limited to day time and relies on the circadian clock. The hypothesis that plasticity in circadian rhythms is functionally significant is supported by the strong link between division of labor and circadian rhythmicity [5, 6, 8], and by comparative studies.

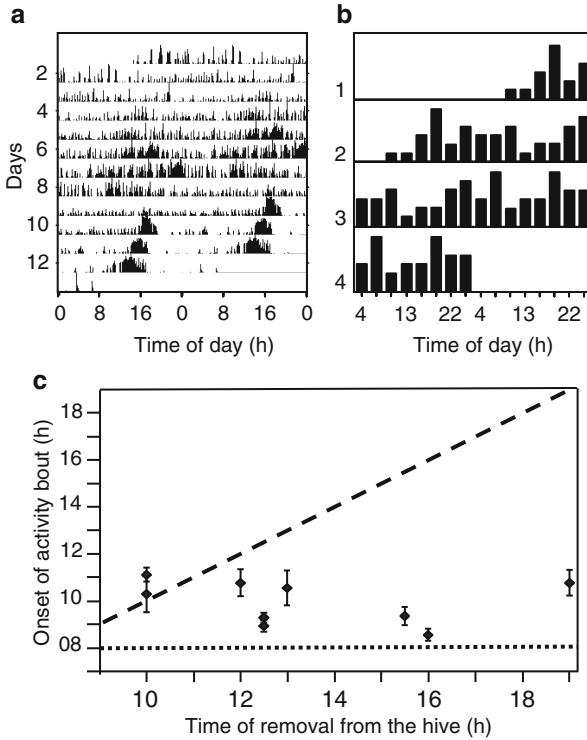


Fig. 1.3.2 Plasticity in circadian rhythms in honey bees (**a**) the ontogeny of circadian rhythms in locomotor activity. A double-plot actogram showing two consecutive days in each row. The height of the black columns in each row corresponds to levels of locomotor activity. A newly-emerged bee was placed individually in a cage in a constantly dark laboratory environment and locomotor activity was monitored automatically (for more details on the data acquisition system see [48]). This bee showed circadian rhythms for the first time on day 9. (**b**) Reversion from activity with, to activity without, circadian rhythms. The double-plot actogram depicts the observed brood care activity of a forager that was induced to revert to nursing behavior (based on data from [5]). (**c**) The onset of the morning bout of locomotor activity for nurse bees removed from the hive. Nurse bees that cared for the brood around the clock in a light–dark illuminated hive were transferred to individual monitoring cages in a constant laboratory environment. Each point shows the onset of the morning bout of activity (mean \pm SE) for nurse bees from one experiment. The *dashed line* depicts a perfect correlation between the time of removal and the onset of morning locomotor activity. The *horizontal dotted line* depicts the onset of the subjective morning (08:00; based on data from [43])

There is a similar task-related plasticity in the bumblebee *B. terrestris* in which division of labor is based primarily on size rather than age as in honey bees and in ants whose division of labor evolved independently of that in honey bees [3, 4].

Plasticity in circadian rhythms is a social behavior and therefore it is important to identify the social signals inducing a bee to switch between activity with and without circadian rhythms. The most straightforward hypothesis is that the brood

regulates activity rhythms because brood care is the main activity of nurse bees and the brood may benefit from being attended around-the-clock. Recent studies in which nurse-age bees were caged on broodless combs inside or outside the hive indicate that plasticity in circadian rhythms is modulated by direct contact with the brood [43]. The identity of the brood signal(s) and the sensory modality by which the signal is detected have yet to be identified.

Another important line of research addresses the mechanisms underlying plasticity in the circadian system. Variation in the environment of nurses and foragers (e.g., light and temperature) cannot account for task-related plasticity in circadian rhythms because nurses are active around-the-clock even when experiencing a light–dark illumination regime, and foragers continue to show strong circadian rhythms under constant conditions [27, 35, 42, 43]. Nurses are typically younger than foragers but their attenuated rhythms are apparently not because their circadian system is undeveloped or underdeveloped. Nurses switch to activity with strong circadian rhythms shortly after transfer to the laboratory, suggesting that their circadian system was capable of generating robust rhythms when they were in the hive [42, 43]. In addition, in colonies with a severe shortage in nurses, some old foragers with strong circadian rhythms revert to care for the brood and are active around-the-clock like nurses in normal colonies [5, 6]. An additional hypothesis is that the molecular feedback loop in brain pacemaker cells in the nurse brain is fixed at a certain state. The molecular and behavioral cycling would take up again from this point when the bee is released from the hive environment. Therefore, if the nurse is removed from the hive, the phase of the oscillations outside the hive would be predicted to be determined by the time of removal. However, when this hypothesis was tested the onset of activity was correlated with the subjective morning in the hive from which the nurses were collected, and not with the time of removal from the hive (Fig. 1.3.2c; [43]). Thus, a more likely explanation is that plasticity in circadian rhythms is mediated by modifications in the functioning or organization of the circadian network. For example, it is possible that some oscillators in the brain of around-the-clock active nurses generate circadian rhythms but these are not synchronized with each other. The oscillators are synchronized again when the nurse is removed from the hive or switches to activities with little or no direct contact with the brood. It is also possible that oscillators in the nurse brain do in fact cycle, but with a low amplitude relative to foragers.

1.3.1.5 Mating-Related Plasticity in Circadian Rhythms of Queens

There is also plasticity in the circadian behavior of queens. Virgin gynes rely on their circadian clock for timing their nuptial flights to a species-specific time of day [22]. Egg-laying queens on the other hand have no diurnal periodicity in behavior [16]. A similar plasticity has been more thoroughly investigated in ants in which the switch to arrhythmicity was shown to be associated with mating; virgin queens that were kept for similar periods, with or without wings, continued to exhibit robust

circadian rhythms [24, 40]. Although plasticity in circadian rhythms of queens is reminiscent of that described above for nurses and foragers, it is probably regulated differently because queens of ants and honey bees do not care for their brood. The functional significance of this behavior may be related to increasing their fecundity, which is critical for the growth and maintenance of their colonies.

1.3.2 Sleep

The circadian clock influences many essential physiological processes, one of which is sleep. In honey bees, it is important to study sleep not only because it is significant for health and functions but also for their behavioral plasticity, remarkable learning capacities, and natural plasticity in circadian rhythms. Surprisingly however, relatively little is known about sleep in honey bees.

1.3.2.1 What Is Sleep?

Three main characteristics are commonly used to define sleep: (1) a period of *quiescence* associated with a specific posture and/or resting place, which is typically accompanied by reduced motor activity, (2) an increased *arousal threshold* (i.e. a higher intensity stimulus is needed to produce a response) and (3) a *homeostatic regulation* mechanism, which is manifested in a sleep rebound after periods of sleep deprivation [45]. A sleep state is distinguished from quiet wakefulness by a decrease in the ability to react to stimuli, whereas the reversibility to an awake state distinguishes sleep from coma [44]. Sleep is regulated by circadian and homeostatic mechanisms which are partly independent. The circadian system plays a crucial role in the timing and consolidation of sleep to an ecologically appropriate period; diurnal animals typically sleep during the night and nocturnal animals during the day [9]. The homeostatic mechanism reflects the need for sleep that accumulates during prolonged periods of wakefulness and dissipates during sustained sleep.

Sleep research has traditionally focused on humans and other mammals. It was commonly thought that true sleep is not found in lower taxa. Over the past three decades studies on diverse non-mammalian species, including fish [31], insects [14, 19, 41, 45], and even nematode worms [32], have shown that rest in these animals meets many criteria of sleep. The molecular pathways associated with sleep in mammals, flies, fish and worms show much conservation, suggesting an ancient and common origin for sleep [1, 9]. Three main areas of molecular conservation in the pathways controlling sleep are the involvement of circadian clock genes (such as *Per*), signaling pathways (such as EGF receptor) and genes involved in neurotransmission (such as GABA receptors) [1].

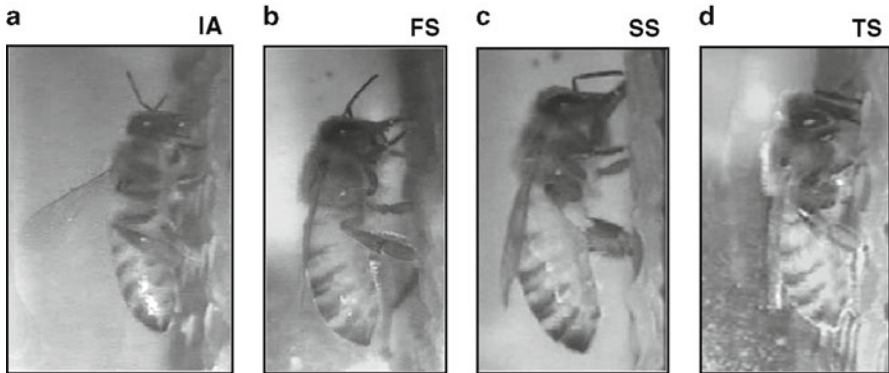


Fig. 1.3.3 Body posture of honey bee workers in various arousal states. Each photograph is a single frame taken from a continuous 24-h video recording. **(a)** Immobile-active state [IA] – an awake bee stays in the same place, the thorax, abdomen, and head are clearly raised above the substrate. **(b)** First sleep stage [FS] – the abdomen and thorax are clearly raised above the substrate, and the antennae are extended at an angle of $90\text{--}180^\circ$, between the pedicle and the scape. **(c)** Second sleep stage [SS] – body is typically more adjacent to the substrate, and the antennae are extended at an angle of $\sim 90^\circ$ between the pedicle and the scape. **(d)** Third sleep stage [TS] – the muscle tonus is reduced, and the body is adjacent to the substrate. The angle between the pedicle and scape $< 90^\circ$, with the antennae tips typically touching the substrate. The three sleep stages also differ in bout duration, antenna movements, and response threshold (From [13])

Although sleep is ubiquitous in the animal kingdom its adaptive value remains an ongoing enigma. Many explanations have been proposed for sleep function, including energy conservation, restoration at the cellular and network levels, maintenance of synaptic homeostasis and memory consolidation (e.g., [26, 34, 44]). Sleep seems to be particularly important for the brain, since the most immediate effect of sleep deprivation is cognitive impairment [10].

1.3.2.2 Do Honey Bees Sleep?

Honey bees are among the first invertebrates for which a sleep-like state was described. In a set of seminal studies, Walter Kaiser and his colleagues characterized the nightly rest behavior of honey bee foragers and proposed that this state shares many behavioral and physiological characteristics of sleep with mammals and birds [18, 19, 36, 37]. Foragers exhibit all three behavioral characteristics of sleep: a period of quiescence, an increased response threshold, and a homeostatic regulation mechanism [18, 23, 36, 37]. Foragers sleep in a *characteristic posture* with relaxed thorax, head and antennae, and with little antennae movements (Fig. 1.3.3). In the hive foragers typically sleep at the periphery of the nest [18, 20]. Both in the hive and in the lab, foragers prefer to rest in locations with an ambient

temperature around 28°C. This preference for a relatively low temperature may allow them to conserve energy since during sleep they are ectothermic and their body temperature is similar to the ambient temperature [38].

Several studies have shown that sleep in honey bees is associated with an increase in **response threshold**. Long-term, extracellular, single-unit recordings from optomotor interneurons in the OLs of honey bee foragers revealed a diurnal oscillation in their sensitivity to moving visual stimuli; the response threshold was higher during the subjective night than during the subjective day [19]. Elevated response thresholds were also found for heat and light stimuli ([13, 18], respectively). For example, the light intensity needed to elicit a response (moving the head and antennae) from a bee in sleep stage three (TS, deep sleep, see Fig. 1.3.3) was about 10,000 times higher than that needed to obtain a similar response from an immobile awake bee [13].

Antennae movement was commonly used as a proxy for honey bee sleep. Kaiser [18] defined sleep as a state of antenna immobility or small amplitude antennal movements. Sauer et al. [37] further showed that this sleep state is dynamic and is correlated with additional characteristics such as a typical head inclination and abdominal ventilatory cycles. Eban-Rothschild and Bloch [13] suggested that honey bee sleep is not uniform and described three different sleep stages (that they termed “First”, “Second” and “Third”; abbreviated FS, SS, and TS, respectively, see Fig. 1.3.3) that differ in body and antennae posture, sleep bout duration, antenna movements, and response threshold. Reduced antennal motility and more pronounced downward tilting of the head, which probably corresponds to deep sleep (TS in [13]) is also associated with an increase in ventilatory cycle duration [37], reduced body temperature, and the low sensitivity (high response threshold) for heat stimuli [18]. In honey bees as in mammals, the transitions from arousal to deep sleep and from deep sleep to awake states are gradual; bees typically enter sleep through the first sleep stage and progress to the second and third stages. These behavioral analyses of sleep dynamics, however, did not find relatively regular sleep cycles with a consistent period as seen in humans and other mammals [13]. There is also one preliminary study suggesting that deep sleep is correlated with rhythmic electrophysiological activity in the mushroom bodies (MBs) [39]. This is an interesting suggestion because the MBs are implicated in sleep in *Drosophila* [17, 30]. In mammals and birds, electroencephalogram (EEG) records, which correspond to neuronal activity in the cerebral cortex, are used for defining sleep and sleep stages. There is also evidence that arousal state is correlated with characteristic electrical brain activities in invertebrates (e.g., [29, 33]). Additional studies are needed to confirm these observations and establish electrophysiological correlates for sleep in bees.

There is evidence that sleep in insects is **homeostatically regulated**, similar to mammals and birds (e.g., [23]). For example, fruit flies exhibit a proportional increase in sleep duration (the index for sleep was continuous bouts of >5 min. with no locomotor activity) following sleep deprivation [14, 41]. Sleep rebound following sleep deprivation in honey bees differed across studies. Hussaini et al. [15] reported that bees that were sleep-deprived for 15 h (during all of the dark phase and some of the light phase), increased sleep duration during the following light phase,

but not during the next dark phase (the index for sleep was the amount of flagella immobility lasting 5 min or more). By contrast, Sauer et al. [36] sleep-deprived foragers for 12 h during the dark phase, and found an increase in sleep (antennae immobility) only during the following dark phase. The latency from the beginning of the dark period to the first episode of antennal immobility (“sleep latency”) tended to decrease following sleep deprivation. These authors also showed that disturbing the bees during the light period (day), did not result in a similar rebound, suggesting that the response was due to sleep lost and not the stress associated with the sleep deprivation procedure [36]. Although these studies differ in the time of sleep rebound (day vs. night), both suggest that bees compensate for sleep lost by intensifying their sleep the following day.

Taken together, the studies reviewed above show that the consolidated nightly rest of honey bee foragers meet the major behavioral and physiological criteria for defining it as sleep.

1.3.2.3 Do Bees That Are Active Around the Clock Sleep?

Because sleep is typically associated with a consolidated period of inactivity, it was not clear whether bees that are active around-the-clock sleep, and if they do, whether their sleep is similar to that of foragers. Two recent studies addressed this question and suggest that around-the-clock active bees do sleep. Young bees that were placed in individual cages in the lab were active around-the-clock but still showed the same three sleep stages as seen in foragers (Fig. 1.3.4). Moreover, the body and antenna postures, antenna movements, and response thresholds for each sleep stage was similar to that in foragers from the same colonies [13]. A precise determination of arousal state is much more challenging in the hive, which is densely populated and where motionless bees may be awake but busy in heat production or brood incubation [21]. Nevertheless, there is evidence suggesting that worker bees, including around-the-clock active cell cleaners and nurses, sleep both inside and outside the comb cells ([20]; sleep was defined as a quiescent state, with no antennae movements for ≥ 3 s). Young honey bees spent more time inside the comb cells, and as they grew older and changed task they tended to sleep more outside the cells. These observations are consistent with previous studies in which the amount of ‘standing motionless’ was recorded for bees performing various tasks in observation hives [28].

Young bees appear to differ from foragers in their sleep dynamics as well. Young bees have fewer sleep bouts during the whole day; however these bouts tend to be longer in comparison to foragers. Foragers tend to progress mainly from light to deep sleep, and from deep sleep they pass directly to awake states, switching less often between sleep stages. Young bees tend to pass more often between the three sleep stages without switching to being awake. It is still unclear whether these differences relate to age or to differences in circadian rhythms between these two groups of bees [13] (Fig. 1.3.4).

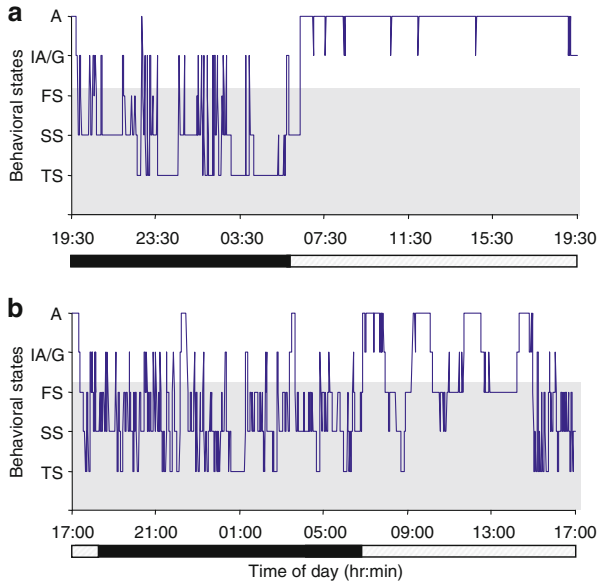


Fig. 1.3.4 Sleep dynamics in honey bees with and without circadian rhythms **(a)** a forager with consolidated activity during the subjective day. **(b)** A young bee that was active around-the-clock. The vertical (Y) axis depicts arousal state (A=active, G=grooming, for other abbreviations see legend to Fig. 1.3.3). *Gray* background indicates that the bee is asleep; *white* background indicates that the bee is awake. The *horizontal bars* at the *bottom* of the plots depict the subjective time. Filled bar=subjective night; open bar=subjective day (From [13])

1.3.2.4 Memory Consolidation During Sleep

In many animals sleep is associated with memory consolidation, the process that transforms new memories to more stable representations that become integrated into the network of pre-existing long-term memories. In both mammals and birds, brain structures implicated in specific learning tasks appear to show the same electrical activity pattern during sleep as during learning (e.g., [11]). Honey bees can provide an excellent model to study the relationships between memory consolidation and sleep, since the functional significance of learning and memory in foraging behavior is well established (see Chaps. 2.5, 6.2, and 6.6) and there has been much progress in understanding the molecular, biochemical and neuronal mechanisms of learning and memory in bees [25]. Surprisingly, this relationship was not explored until recently.

Hussaini et al. [15] tested the influence of sleep deprivation on the acquisition and extinction of new memories. They conditioned foragers to associate odors with a food reward and tested their memory for this association at various time intervals. Sleep deprivation had no effect on memory acquisition, but significantly reduced extinction learning. These findings are consistent with studies in mammals in which sleep deprivation impaired performance in some learning paradigms but not in others.

1.3.3 Outlook

The circadian clock of the honey bee is involved in complex behaviors such as sun-compass orientation, time-memory, division of labor, and social coordination of worker activities, all of which can be studied in a relatively natural context. Recent studies on time memory have shown that there is still much to learn even about circadian behaviors, which were described years ago in bees (reviews: [3, 4]).

The circadian clock is emerging as an important element in the temporal coordination of honey bee society. The circadian system of honey bees is very sensitive to social influences and shows remarkable plasticity. These characteristics may have been shaped by social evolution [3, 4]. The hope is that future studies will discover the specific social signals and the sensory modalities by which the social environment modulates the circadian behavior of bees. Unveiling the molecular and neuronal bases of plasticity in circadian rhythms is another important line of future research, with possible implications that are far beyond the sociobiology of bees. Another line of socio-chronobiological research that should be pursued in the future is social entrainment [3, 4].

Their social behavior, chronobiological plasticity, and remarkable learning capacities also make honey bees an attractive model to study sleep in a natural context. For example, foragers orient themselves over long distances and rely more heavily than nurses on visual learning. This natural variability between nurses and foragers that develop in a similar hive environment and are genetically related creates a natural model system with which to study the adaptive value of sleep, which has been commonly hypothesized to be linked to memory consolidation and synaptic plasticity. In order to effectively study honey bee sleep, protocols for sleep deprivation need to be developed, including in the hive. It is also needed to develop methods for precisely recording sleep in bees in the complex social environment of the hive.

We need to know much more about the neuronal and molecular mechanisms governing circadian rhythms and sleep in honey bees. This is particularly true for sleep, which has not yet been correlated with specific genes, anatomical structures or neurophysiological processes. There are also significant gaps in our knowledge of the molecular biology of the circadian clock, and the neuroanatomical characterization of the circadian network is only at its very initial stages. Molecular and neurobiological studies on sleep and circadian rhythms will not only help enhance our understanding of these important systems, but will also set the stage for studies on the ways these systems interact with each other and affect the social and foraging behavior of bees.

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