

Circadian plasticity in honey bees

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Circadian rhythms of about a day are ubiquitous in animals and considered functionally significant. Honey bees show remarkable circadian plasticity that is related to the complex social organization of their societies. Forager bees show robust circadian rhythms that support time-compensated sun-compass navigation, dance communication and timing visits to flowers. Nest-dwelling nurse bees care for the young brood around the clock. Here, we review our current understanding of the molecular and neuroanatomical mechanisms underlying this remarkable natural plasticity in circadian rhythms.

Introduction

Our planet completes a rotation around its axis every 24 hours creating day–night oscillations in many environmental variables. Many organisms have evolved circadian clocks, enabling them to anticipate these predicted changes and align their physiology and behaviour to their constantly changing environment. Perturbations to normal clock functions reduce performance and increase the risk of illness. Circadian clocks are endogenous, meaning that they tick even without environmental signals, but their phase is repeatedly reset by ecologically relevant time cues such as sunlight, food consumption and temperature cycles. The circadian clock influences many downstream molecular, biochemical and physiological processes in numerous cells and tissues throughout the body. Given the importance of normal circadian rhythms to animal health and performance, it was quite perplexing to discover that honey bees and other social insects can show relatively extended periods of activity around the clock with no circadian rhythms and no apparent ill-effects.

Honey bees live in complex societies (known as ‘advanced eusociality’) typically consisting of a single reproductive ‘queen’, several tens of thousands of non-reproducing ‘worker’ bees that are typically the queen’s daughters and up to several hundred males. They show elaborate communication systems, allowing them to coordinate almost any aspect of their life. Worker bees generally specialize in certain tasks, forming a division of labour that is related to their age. Young workers usually care for (nurse) the brood and the queen, at later ages they perform nest maintenance activities and at the age of about three weeks, they switch to outside activities such as nest guarding and foraging for resources such as nectar, pollen, water and propolis.

Circadian rhythms in bees

The honey bee was one of the first models for research on circadian rhythms. By the early 1900s, it had been discovered that honeybees have a ‘time sense’, allowing them to visit flowers at times of highest reward availability. Later

studies have implicated the circadian clock in additional complex behaviours such as sun-compass orientation and dance communication, which use directional information relative to the sun location above the horizon. The bees consult their circadian clock to compensate for the sun movement during the day. Given the importance of this clock for successfully foraging and communicating the location of floral resources, it is not surprising that foragers have strong circadian rhythms with activity during the day and sleep during the night. This remains true even when isolated under constant laboratory conditions, indicating that the rhythms are endogenous. Only in the late 1990s it was discovered that strong circadian activity rhythms are not typical to all the bees in a colony.

Socially mediated plasticity in circadian rhythms

When individually tagged bees were placed in observation hives with transparent glass walls, it was discovered that nurse bees tend larvae around the clock without daily activity cycles. It is assumed that around-the-clock care improves brood development, enabling faster colony growth. The premise that task-related division of labour is adaptive is supported by comparative studies showing similar patterns in bumble bees, in which division of labour relates to body size rather than to age, and in ants in which sociality has evolved independently of bees. Remarkably, there is substantial plasticity in division of labour. For example, when there is a shortage of foragers (e.g. due to high predation outside the nest), some young worker bees mature faster and start foraging at an early age compared to typical colonies. If there is a shortage of nurses, some of the older foragers revert to care for the brood (Figure 1). This remarkable plasticity in circadian rhythms is regulated by contact with the brood. Workers in the hive, in small groups or individually isolated, can be induced to switch to activity around the clock if placed with pupae or larvae, and switch to strong circadian rhythms if separated from the brood. Workers that are enclosed in a mesh cage on a broodless piece of wax comb show strong circadian rhythms even if they can smell the odour of larvae.

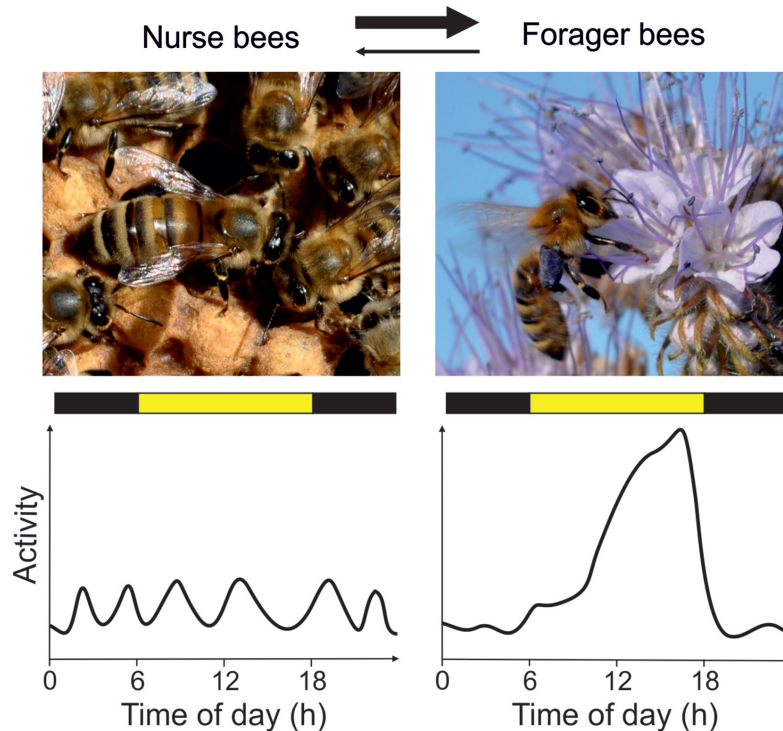


Figure 1. Socially regulated plasticity in the circadian activity of honey bees. Nurse bees care for the brood without daily rhythms, whereas foragers are typically more active during the day and sleep at night. Division of labour is plastic and socially regulated. Young bees develop circadian rhythms with age (thick arrow). However, under certain conditions such as a shortage of young nurses, foragers can revert to nursing (thin arrow). The yellow and black bars on top of the activity plots indicate day- and night-time, respectively.

We now know that extended periods of activity around the clock, and plasticity in circadian rhythms, are not limited to bees and other social insects. For example, it is common in Arctic birds and mammals during the long, 24-hour light, summer days. Marine mammals need to breathe air at the sea surface, and many open-sea fishes such as tuna and some sharks need to constantly move in order to get sufficient flow of oxygen over their gills. Given that the molecular circadian 'clockwork' (i.e. the biochemical 'cogs' driving the clock) is similar in animals as diverse as mammals and insects, it is not clear why some animals can be active around the clock with no apparent ill-effects, while others like humans, suffer from perturbations to the normal rhythms of their body. Studies with bees have started to explore the molecular and neuronal mechanisms underlying natural plasticity in circadian rhythmicity.

The molecular clockwork of bees

Many clock-related genes are known. The honey bee genome encodes the mammalian-type *Cryptochrome* (*Cry-m* or Insect *Cry2*) but not *Timeless1* and the *Drosophila*-type *Cryptochrome* (known as Insect

Cryptochrome 1 [*Cry1*], or *Cryptochrome-d* [*Cry-d*]), which are essential components of the fly clockwork. Consistent with these findings, additional bioinformatics and expression analyses suggest that the honey bee clockwork is more similar to that of mammals than to *Drosophila* flies. The molecular clockwork is based on transcriptional-translational feedback loops. In the honey bee model, the proteins encoded by the *Cycle* (*Cyc*) and *Clock* (*Clk*) genes interact with E-box sequences in the promoters of *Cry-m* and *Period* (*Per*) and activate their transcription. The translated PER and CRY-M proteins are translocated into the nucleus, in which they inhibit the transcriptional activity of the CLK:CYC protein complex. This negative feedback loop downregulates the transcription of *Per* and *Cry-m* (Figure 2). In the bee, as in mammals, it appears that *Cyc* functions in a 'positive' loop that is thought to stabilize the clockwork. This is different from *Drosophila* in which *Clk* forms the positive loop. The circadian expression patterns of additional known clock genes of the positive loop, such as *Par domain protein 1* and *Vrille*, have not yet been studied with sufficient detail in honey bees. Given that in *Drosophila*, *Tim1* and *Cry-d* are necessary for light resetting of the clock, their absence from the honey bee genome suggests that bees use different light

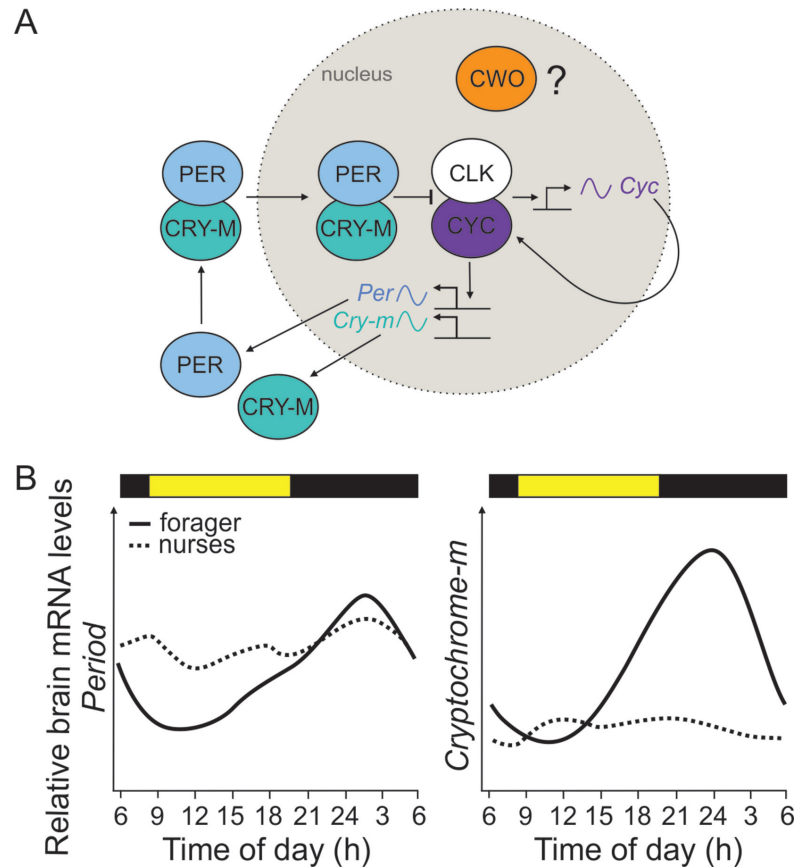


Figure 2. The molecular clockwork of the honey bee. **A**) A model for the circadian clockwork in the honey bee brain. The Cycle (CYC) and Clock (CLK) proteins form a complex that activates the transcription of the *Period* (*Per*) and *Cryptochrome-m* (*Cry-m*) genes. Their translated proteins are translocated into the nucleus in which they inhibit the transcriptional activity of the CYC:CLK protein complex. This down-regulates their own expression, forming a negative transcriptional/translational feedback loop. *Cyc* is part of a second feedback loop that is thought to stabilize the molecular clockwork and is not yet characterized in detail in bees. *Clockwork Orange* (*Cwo*) shows strong oscillations in the bee brain and may have a similar transcriptional repression function as in *Drosophila*. **B**) Whole brain transcript abundance of the clock genes *Per* and *Cry-m* oscillate in foragers but not in nurses. The yellow and black bars are as in Figure 1. A similar pattern of mRNA levels over the day is seen for foragers and nurses, which are collected after the bees experienced constant conditions, indicating that the pattern is endogenous and not driven by the light–dark illumination regime.

input pathways. *Clockwork orange* (*Cwo*), which represses transcription in *Drosophila*, cycles with a similar phase in the bee brain, consistent with the premise of a similar function. The characterization of the molecular clockwork of the bee sets the stage for comparing its function in foragers that show circadian rhythms and nurses that do not.

Measurements of clock gene transcript abundance in the brain of foragers reveal robust circadian oscillations for *Per*, *Cry-m*, *Cyc*, *Cwo* and *Timeout* (a paralog of *Tim1* with no known role in the circadian clock), as can be expected for an animal showing strong activity rhythms. In similar analyses in sister nurse bees on the other hand, the same genes show no, or at best weak, oscillations in transcript abundance. These findings may suggest that the nurse clock stops ticking. However, nurses show strong

circadian rhythms in locomotor activity and clock gene expression shortly after transfer from the hive (or brood-containing cages) into a constant laboratory environment. Moreover, the phase of their circadian rhythms is aligned with the day–night cycles outside the hive. How can clocks of nurses inside the dark and thermoregulated hive be in synch with ambient day–night cycles? Studies using several experimental approaches indicate that nurse bees are socially synchronized by foragers that are exposed to the environment outside the nest. Remarkably, when nurses experiencing conflicting social and light–dark cycles are removed from the hive and monitored in constant condition, they show circadian rhythms with a phase more similar to the social cycle. Thus, the honey bee is the first animal for which social synchronization was shown to override synchronization by light. How can this

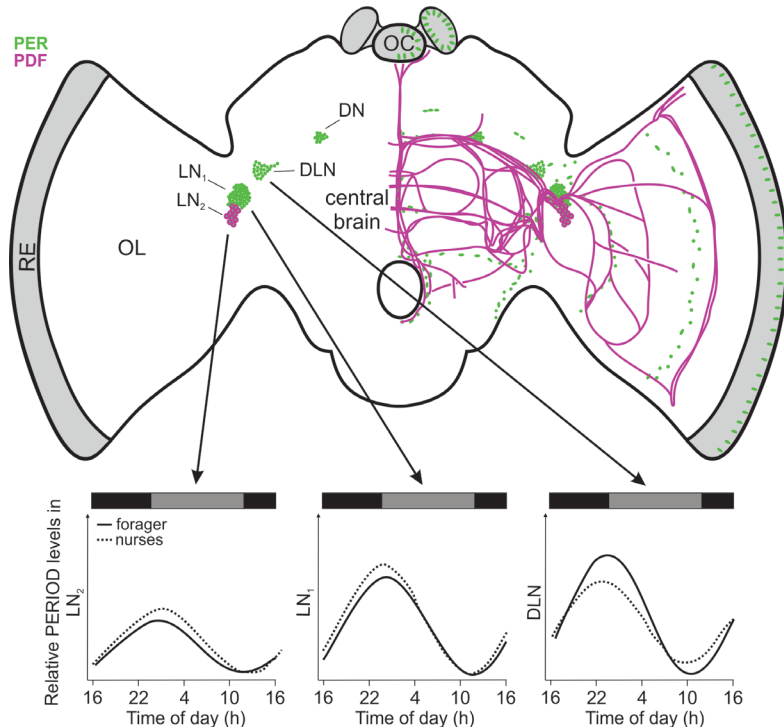


Figure 3. Neuronal clock protein oscillations in behaviorally rhythmic foragers and arrhythmic nurses. **Top.** Schematic organization of the circadian neuronal network in the honey bee brain. Green: cells expressing the clock gene PERIOD protein (PER); purple: cells expressing the circadian neuropeptide Pigment Dispersing Factor (PDF). The left hemisphere shows only the cell bodies of clock neurons. The LN_2 cluster expresses both PER and PDF. The right hemisphere shows the highly complex arborization network of PDF positive neurons as well as PER expressing glia cells. OC: ocelli; OL: optic lobe; RE: retina. **Bottom.** PER levels in clock cell clusters LN_2 , LN_1 and DLN similarly cycle in nurses and foragers collected under constant conditions. The grey and black bars on top of the graphs, respectively, depict day- and night-time outside the hive.

evidence for phase resetting be reconciled with their lack of circadian rhythms in behaviour and whole brain clock gene expression? To answer this question, it is necessary to better understand the neuroanatomical organization of the circadian network in the honey bee brain.

Neuronal organization of the bee clock

To this end, the neuroanatomical description of the honey bee circadian clock network is based on immunostaining using antibodies specifically recognizing the core clock protein PER and the circadian neuropeptide Pigment Dispersing Factor (PDF). The honey bee clock network consists of several neuronal clusters centered in the dorsal and lateral brain (Figure 3). PER is also expressed in non-neuronal glia cells that are scattered throughout the brain. The neuronal organization of the circadian clock is similar to that of other insects consistent with a common ground plan of the insect circadian system. The LN_2 is the only cluster in which PER and PDF are co-expressed. These neurons build a highly complex arborization network (Figure 3), connected with

many parts of the brain, including neuroanatomical structures regulating locomotion and endocrine systems. As neurons of the clock network also overlap with sun-compass pathway neurons, they may be involved in the integration of circadian and sun-compass information that is necessary for time-compensated sun-compass orientation and waggle dance communication.

Quantification of PERIOD protein levels in clock neuronal clusters revealed strong and similar circadian oscillations in behaviourally rhythmic foragers and arrhythmic nurses. The oscillations in the nurse bees are not consistent with their attenuated cycling in whole brain *Per* mRNA levels. One possible explanation for this apparent discrepancy is task-related variability in clock gene expression in glia cells that are included in whole brain analyses, but not in PER protein quantification that was limited to neurons. The oscillations in clock neurons lend credence to the behavioural studies suggesting that around-the-clock active nurse bees in the tightly regulated nest environment nevertheless have functional clocks that measure time and can be synchronized with environmental time cues.

Summary

The honey bee provides an excellent model for studying the interplay between the circadian clock and complex behaviours including those related to their elaborated social life style. The molecular clockwork of the bee is in many ways more similar to mammals than to fruit flies. Honey bees naturally switch between activity with and without circadian

rhythms along with the task they perform. Nurse bees that are active around the clock nevertheless have a functional clock that is socially synchronized by the activity of rhythmic foragers. The temporal pattern of whole brain clock gene transcript abundance differs between rhythmic foragers and arrhythmic nurses suggesting that plasticity in circadian rhythms is mediated by functional modifications in the brain circadian clock system. ■

Further reading

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