ANNUAL REVIEWS

Annual Review of Entomology

Sleep in Insects

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Annu. Rev. Entomol. 2018. 63:69–86

The *Annual Review of Entomology* is online at ento.annualreviews.org

https://doi.org/10.1146/annurev-ento-020117-043201

Keywords

genetic and neuronal basis, dopamine, mushroom body, central complex, pars intercerebralis, pars lateralis, circadian clock

Abstract

Sleep is essential for proper brain function in mammals and insects. During sleep, animals are disconnected from the external world; they show high arousal thresholds and changed brain activity. Sleep deprivation results in a sleep rebound. Research using the fruit fly, *Drosophila melanogaster*, has helped us understand the genetic and neuronal control of sleep. Genes involved in sleep control code for ion channels, factors influencing neurotransmission and neuromodulation, and proteins involved in the circadian clock. The neurotransmitters/neuromodulators involved in sleep control are GABA, dopamine, acetylcholine, serotonin, and several neuropeptides. Sleep is controlled by the interplay between sleep homeostasis and the circadian clock. Putative sleep-wake centers are located in higher-order brain centers that are indirectly connected to the circadian clock network. The primary function of sleep appears to be the downscaling of synapses that have been built up during wakefulness. Thus, brain homeostasis is maintained and learning and memory are assured.

INTRODUCTION

Sleep is a fascinating behavioral state whose function still remains unclear. Humans spend about one-third of their life sleeping, and many other mammalian species such as (o)possums (*Didelphis marsupialis*, *Lutreolina crassicaudata*, *Trichosurus vulpecula*), kangaroos (*Megaleia rufa*) or kangaroo rats (*Potorous apicalis*), tree shrews (*Tupaia glis*), hedgehogs (*Erinaceus europaeus*, *Paraechinus hypomelas*), bats (*Eptesicus focus*, *Myotis lucifugus*), beavers (*Aplodontia rufa*), chipmunks (*Tamias striatus*), golden hamsters (*Mesocricetus auratus*), gerbils (*Meriones unguiculatus*), and rats (*Rattus norvegicus*) spend more than half of their life sleeping (19, 134). Although short sleepers such as elephants (*Elephas maximus*, *Loxodonta africano*), horses (*Equus caballus*), donkeys (*E. asinus*), tapirs (*Tapirus terrestris*), cows (*Bos taurus*), and sheep (*Ovis aries*) show consolidated sleep periods of only 3–5 h per 24-h cycle, sleep nevertheless appears to be an essential part of animal life. In the sleep state, animals are disconnected from the external world, owing to elevated sensory thresholds, at considerable risks and costs to the individuals. During sleep, animals cannot forage or take care of their young. Nor can they escape from potentially life-threating situations. Thus, there must be a vital benefit of sleep. Indeed, a life without sleep seems impossible. Prolonged sleep deprivation in rats results in death (102). Also, humans with fatal familial insomnia eventually die within one to two years (30). Humans present impaired cognitive performance after only one day of sleep deprivation, and longer sleep deprivation results in hallucinations and alalia (speech delay), indicating the importance of sleep for proper brain function.

Sleep is similarly important for invertebrates, including insects. In honey bees (*Apis mellifera*), sleep deprivation at night impairs the precision of waggle dance signaling (73) and reduces the probability of successfully returning to the hive the following day (11). In fruit flies (*Drosophila melanogaster*), sleep deprivation causes defects in short- and long-term memory (114, 115) but significantly improves learning and memory (35, 39). Because the insect brain is simpler than a mammal's brain, the study of sleep in insects promises new insights into its neuronal basis and functional role. Here, I review our current understanding of sleep in insects.

DEFINITION OF SLEEP

Sleep can be reliably defined in terms of behavioral criteria: Animals adopt a species-specific sleep posture in which they move little or not at all. Their sensory thresholds are elevated, but sleep is reversible with stronger stimulation. In vertebrates, the basis for such a behavioral definition of sleep was provided by Pirron as early as 1913 (99) and elaborated by Flanigan in 1972 (44). In 1983, Tobler (131) proposed that the homeostatic nature of sleep should be considered as an additional functional criterion of sleep. Sleep need continuously increases during wakefulness and is reset to zero only after proper sleep. Consequently, sleep deprivation leads to a compensatory increase of sleep, or sleep rebound (131–133). Nowadays, the homeostatic nature of sleep is regarded as one of the most important sleep criteria.

HISTORY OF SLEEP STUDIES IN INSECTS

Sleep-like states have been described as early as 1912 for different insects such as bees (Apidae), wasps (Vespidae), moths (Pyralidae), mosquitos (Culicidae), and cockroaches (Blattoidae) (4, 43, 54, 67–70, 111, 131). They were also demonstrated for scorpions (Scorpionidae) (136) and crayfish (*Procambarus clarkii*) (101). All these arthropods choose explicit sleeping places and adopt specific sleep postures when sleeping, as shown for different hymenopteran species, cockroaches (Blattidae), and flies (Drosophilidae) (**Figure 1**). In honey bees, sleep can also be unequivocally deduced from the position of their antennae (**Figure 2**) (67, 69, 111).

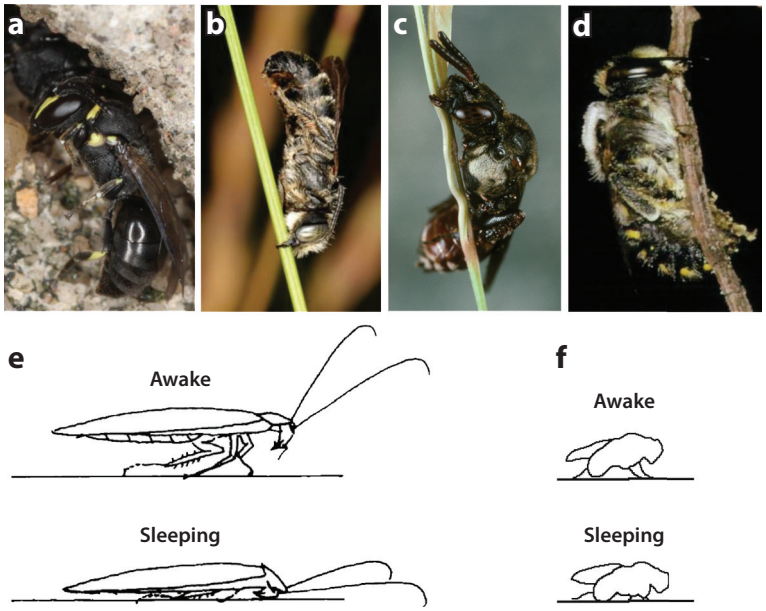

Figure 1

Sleep postures of different insects. (*a*) *Hylaeus communis*. (*b*) Unknown solitary bee. Panels *a* and *b* from G. Erbe; **http://www.guntramerbe.de/maskenbienen/Erbe_Schlafplatzwahl_2016.pdf**. (*c*) *Biastes emarginatus* (145). (*d*) *Anthidium septemspinosum* (55). (*e*) Cockroach (*Blaberus giganteus*) (modified from Reference 135). (*f*) Fruit fly (modified from Reference 62).

While asleep, insects not only are immobile and show a high arousal threshold, but also reduce the responsiveness of their nervous system to external stimuli. This finding was demonstrated first for the honey bee by Kaiser & Steiner-Kaiser (70). In sleeping bees, optomotor interneurons in the lobula did not respond to moving patterns presented to their eyes, whereas each movement provoked a clear electrical response when bees were awake. Air puffs delivered to the mechanosensory

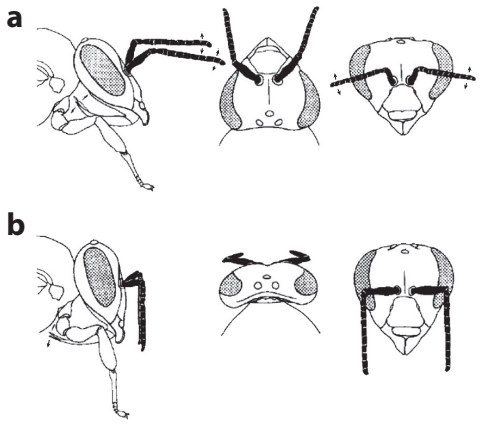

Figure 2

Position of the antennae in *Apis mellifera* during (*a*) wakefulness and (*b*) sleep (modified from Reference 68).

hairs around the eye temporarily woke up the bees, enabling the neurons to respond briefly to the moving pattern, most likely owing to stress-dependent octopamine release (70, 105, 109). Even the homeostatic nature of sleep was shown for several insect species: Sleep deprivation resulted in reduced alertness and in sleep rebound during recovery (13, 108, 131, 135, 136).

Despite increasing evidence that sleep in insects resembles sleep in mammals and birds, the sleep research community did not easily accept this view. Only after sleep research in *D. melanogaster* demonstrated that orthologous genes were activated during sleep in mammals and fruit flies (62, 119) did sleep in insects gain acceptance.

Fruit flies fulfill all criteria for sleep as defined above: They exhibit a species-specific sleep posture (**Figure 1**). They show consolidated periods of immobility, increased arousal threshold during these periods, and a significant sleep rebound after sleep deprivation (62, 119). In addition, electrical brain activity correlates with the activity state of the fruit fly (91). Local field potentials recorded in the medial brain of living fruit flies in concert with their leg movements showed that periods of longer immobility were associated with decreased incidence of spike-like neuronal activity. Sleep in fruit flies appears to comprise stages of varying sleep depth (141), and Ca^{2+} levels in many cells of the *Drosophila* brain decline during sleep and increase during wakefulness (18). Thus, sleep in *D. melanogaster* is accompanied with changes in brain activity. Perhaps even more interesting is that sleep need in *D. melanogaster* changes with aging. Similar to humans, young fruit flies sleep significantly longer than old fruit flies and sleep becomes fragmented with age (74). In addition, short-sleeping fruit fly mutants appear to have a reduced life span (16). The fruit fly has quickly developed as a model by which to understand the molecular and neuronal mechanisms of sleep, and large parts of this review are therefore based on results from research using *D. melanogaster* as a model system.

THE GENETIC AND MOLECULAR BASIS OF SLEEP

Genes that affect sleep across species can be categorized as genes that (*a*) code for ion channels, (*b*) affect neurotransmission and neuromodulation, and (*c*) affect circadian rhythms (26). I highlight a few of the genes belonging to the first two categories and discuss the interplay between sleep and the circadian clock.

Ion Channels

Shaker and *Hyperkinetic* were among the first genes in *D. melanogaster* identified by mutagenesis screens (15, 28). The two genes code for different subunits of a voltage-gated potassium channel (K^+) responsible for the control of membrane repolarization and transmitter release. *Shaker* and *Hyperkinetic* mutants have an overall increased neuronal excitability and a short-sleeping phenotype. Null mutants of the *Shaker* gene such as *minisleep* sleep only 2–4 h per day instead of the normal 8–10 h. The second gene with strong effects on sleep duration is *sleepless* (75). Like *Shaker* mutants, *sleepless* mutants only sleep ~2 h per day. *Shaker* and *sleepless* interfere with each other (148, 149): SLEEPLESS is a Lys-6 glycosylphosphatidylinositol (GPI)-linked protein that promotes *Shaker* expression and activity. SLEEPLESS appears to inhibit acetylcholine (ACh) receptors and thus affects excitatory neurotransmission (see below).

Voltage-gated potassium channels (Kv) are also involved in mammalian sleep control. Mice lacking the closest mammalian homolog of *Shaker*, *Kv1.2*, sleep less than wild-type mice do, but their sleeping phenotype is less dramatic than that in fruit flies, probably because of the redundant functions of *Kv* genes in mammals (152). Furthermore, a mutation in an ATP-sensitive K^+ channel subunit, which serves as a sensor for intracellular energy metabolism, renders humans and fruit flies short sleeping (3).

Neurotransmission and Neuromodulation

As stated above, *sleepless* interferes with neurotransmission through ACh by blocking nicotinic ACh receptors. ACh, catecholamines, histamine, and serotonin are the wake-promoting neurotransmitters in mammals. Except for serotonin, the same neurotransmitters promote wakefulness in fruit flies. Wild-type *sleepless* diminishes the wake-promoting effect of ACh. The *sleepless* mutants lack this inhibition and therefore are more awake (113, 148). In contrast, the sleep mutant *redeye* carries a mutation in a specific nicotinic ACh receptor subunit, has consequently reduced ACh signaling, and sleeps more (122). All this accords with the wake-promoting effect of ACh.

The strongest wake-promoting neuromodulators in mammals and fruit flies are probably catecholamines (e.g., dopamine, norepinephrine, and octopamine). Hyperactive and sleepless *fumin* mutants of *D. melanogaster* carry a mutation in the dopamine transporter that transports released dopamine back into the dopaminergic neurons (78). The mutated dopamine transporter only partly fulfills this function, which consequently leads to permanently high dopamine levels in the synaptic cleft that continue to activate dopamine receptors on the postsynaptic neurons. Similar wake-promoting and sleep-reducing effects are observed after dopaminergic neurons are activated, which leads to continuous dopamine release (83, 118). Conversely, mutants deficient for tyrosine hydroxylase, the rate-limiting enzyme for dopamine synthesis, have reduced dopamine levels and increased sleep throughout the day (104). Octopamine is the insect homolog of norepinephrine. Similar to norepinephrine in mammals, octopamine has strong wake-promoting effects in fruit flies: Mutations in the enzymes responsible for synthesizing octopamine or silencing octopaminergic neurons increase daily sleep amount, whereas activating octopaminergic neurons decrease sleep (31, 113).

The wake-promoting neuropeptides in fruit flies are diuretic hormone DH31 (79), which is homologous to the mammalian calcitonin gene-related peptide that increases locomotion in zebrafish (*Danio rerio*) (147); diuretic hormone DH44 (20), which is homologous to the mammalian corticotropin-releasing factor that prepares mammals for activity and stress; and *D. melanogaster* insulin-like peptides (DILPs) (32), which regulate sugar levels in the blood and hemolymph and influence growth, metabolism, and reproduction in all animals. The activity-promoting factor adipokinetic hormone (AKH) is a functional analog of glucagon, which mobilizes energy reserves from storage sites and stimulates activity (146). In *D. melanogaster*, AKH induces starvation-dependent hyperactivity (81). Finally, leucokinin stimulates fruit fly activity after feeding (i.e., reduces postprandial sleep) (90) and neuropeptide F (NPF) promotes wakefulness and feeding (26).

The sleep-promoting neurotransmitters/neuromodulators in fruit flies are GABA (γ-aminobutyric acid) (2, 51, 86, 94) and to some extent serotonin (56, 153). Sleep-promoting neuropeptides include epidermal growth factor receptor (EGFR) (47), which regulates circadian activity in mammals (76), AYRKPPFNFSIFamide (SIFamide) (96), short neuropeptide F (sNPF) (24, 116), and allatostatin A (AstA) (23). The fact that SIFamide (87), and AstA (23) affect feeding in addition to their role in sleep promotion indicates a strong link between the metabolic state of the fruit fly and sleep. An increasing number of other neuropeptides that influence sleep are not mentioned in this review.

Octopamine Does Not Increase Compensatory Sleep

Homeostasis is an important aspect of sleep. However, not all sleep-loss-provoking manipulations in the fruit fly lead to sleep rebound. This is especially true for sleep loss provoked by octopamine (113): Although fruit flies are aroused and unable to sleep when octopaminergic neurons are thermogenetically activated at night, they do not require additional sleep the following morning when they are returned to normal temperatures. The same seems true for sleep loss provoked

by starvation and other stressors (129, 130). Thus, there are situations in which fruit flies can be active (even at night) without provoking additional sleep need and memory impairments. Such high activity is essential for escaping from stressful situations or during migration: Several birds and insects [e.g. thrushes (*Catharus guttatus*, *C. ustulatus*), godwits (*Limosa limosa*), garden warblers (*Sylvia borin*), dragon flies (*Pantala flavescens*), and locusts (*Chortoicetes terminifera*)] migrate during their normal sleep episodes, which prevents them from sleeping, without obvious effects on their health and performance (34). In *D. melanogaster*, stress response genes appear to protect against the lethal effects of sleep deprivation (120). Thus, sleep deprivation can be uncoupled from sleep need under certain circumstances; octopamine and putatively DH31, DH44, and AKH appear to be arousal factors that do not affect sleep homeostasis. This does not apply to dopamine and ACh: Activating dopaminergic or cholinergic neurons during the night leads to a sleep rebound the following morning (39, 40, 113, 123).

Differential Expression of Genes During Sleep and Wakefulness

Whereas some genes affect sleep, other genes are differentially expressed during sleep and wakefulness as revealed by whole-genome transcriptomic studies (reviewed in 27). Sleep- and wakefulness-related transcripts belong to different functional categories, suggesting that the two behavioral states favor different cellular processes, and this scenario is true for mice (*Mus musculus*), sparrows (*Zonotrichia leucophrys gambelii*), and fruit flies (*D. melanogaster*).

Typical wakefulness-related genes mobilize energy stores, respond to cellular stress, and facilitate synaptic potentiation. Typical sleep-related genes are involved in protein synthesis, lipid metabolism, and synaptic consolidation or downscaling. In sum, energy-consuming synaptic potentiation is favored during wakefulness, whereas protein synthesis required by synaptic consolidation and membrane lipid trafficking occurring during synaptic depression prevail during sleep.

SLEEP IS CONTROLLED BY THE INTERPLAY BETWEEN SLEEP NEED AND THE CIRCADIAN CLOCK

Sleep is controlled by two processes: a homeostatic drive that increases during wakefulness and dissipates during sleep, and a circadian pacemaker that controls its timing (12). Both processes work together and determine the occurrence of consolidated sleep at an ecologically appropriate time.

Disturbances to the circadian clock leave the homeostatic control of sleep largely intact: Fruit flies carrying null mutations in circadian clock genes (63) no longer consolidate sleep in one major phase but distribute it among many bouts of sleep. Conversely, manipulations of baseline sleep leave the general daily timing of sleep intact. For example, substances such as caffeine or antihistamines alter sleep duration or latency but do not affect global sleep timing (27, 62, 119). The same scenario applies for most of the abovementioned sleep mutants. They sleep significantly more or significantly less, but their sleep is consolidated in one major phase.

The Influence of the Circadian Clock on Sleep Is Highly Plastic

The influence of the circadian clock on sleep-wake cycles is modulated by factors such as metabolic state, social interactions, stress, and environmental conditions. Hunger can induce animals to search for food during their usual sleeping time, and satiety can promote postprandial sleep even during their usual wake time (see above) (72, 90, 126). Animals can even invert their sleep-wake cycles when necessary. For example, wild pigs (*Sus scrofa*) switch from diurnal to nocturnal activity

when hunted extensively and revert to diurnality when undisturbed (142). Nocturnal animals such as mice (*M. musculus*) can become diurnal in cold winters to save energy (142). Similarly, certain ant species (*Camponotus* spp.) are nocturnal in summer and diurnal in winter (33), and diurnal fruit flies (*D. melanogaster*) can switch to partial nocturnality when the nights are illuminated by dim light or the environmental temperature is high (7, 95). Social interactions influence sleep time: Daytime sleep increases in fruit flies exposed to socially enriched environments (49), and *Drosophila* males become night active when many of them are housed together (48). Thus, the clock's control of sleep is not hardwired. Indeed, the connection between the circadian clock and the sleep-wake centers is indirect (see below), allowing animals to adapt their sleep-wake cycle to internal and external needs.

THE NEURONAL BASIS OF SLEEP

Specific brain areas in mammals and fruit flies control wakefulness and sleep and their daily timing (reviewed in 41, 107). In the following section, I briefly describe these areas of the mammalian brain; however, I provide a more detailed description for the insect brain.

Circadian Clock and Sleep Centers in the Mammalian Brain

The mammalian circadian clock is located in the suprachiasmatic nuclei (SCN) of the hypothalamus. It is indirectly connected to the sleep and wake centers in the hypothalamus, basal forebrain, and brain stem. The SCN sends projections into the ventral subparaventricular zone of the hypothalamus, which in turn sends projections into the dorsomedial nucleus of the hypothalamus, from which projections run to the sleep and wake centers (reviewed in 107). Wake- and sleep-promoting neuronal groups interact in the control of sleep. The wake-promoting neuronal groups comprise dopaminergic, noradrenergic, cholinergic, histaminergic, and serotonergic neurons, whereas the sleep-promoting neuronal groups express the inhibitory neurotransmitter GABA. Sleep- and wake-promoting neuronal groups mutually inhibit each other. This enables a switch between the two systems that depends on sleep need and signals from the circadian clock (106).

Circadian Clock and Sleep Centers in the Insect Brain

The insect circadian clock is located in the accessory medulla (AME), a small neuropil at the base of the second optic ganglion, the medulla (60, 103). The mammalian SCN and the insect AME show astonishing functional similarities (58, 127) and differ mainly in morphological details, such as the arrangement of clock neuron somata. Whereas the clock neuron somata are contained in the SCN, they are located outside the AME in insects. This display is most conspicuous in *D. melanogaster*, in which the somata are distributed throughout the dorsal and lateral brain, sometimes far away from the AME (**Figure 3**). Nevertheless, virtually all fruit fly clock neurons send fibers into the AME. In addition, almost all clock neurons send fibers into the dorsal brain (i.e., superior protocerebrum), where these terminate close to higher-order centers of the *Drosophila* brain such as the mushroom bodies (MBs), the central complex (CX), and the neurohormonal centers in the pars intercerebralis (PI) and pars lateralis (PL) (**Figure 3**). Exceptions are the so-called large ventrolateral neurons (l-LN_v), which do not project dorsally but appear to signal to the other clock neurons via the neuropeptide pigment-dispersing factor (PDF) released into the AME (42, 59, 110).

All the abovementioned higher-order brain centers were suggested to contain sleep-wake centers of the fruit fly. The MBs were the first neuropils to be considered as critical regulators of sleep

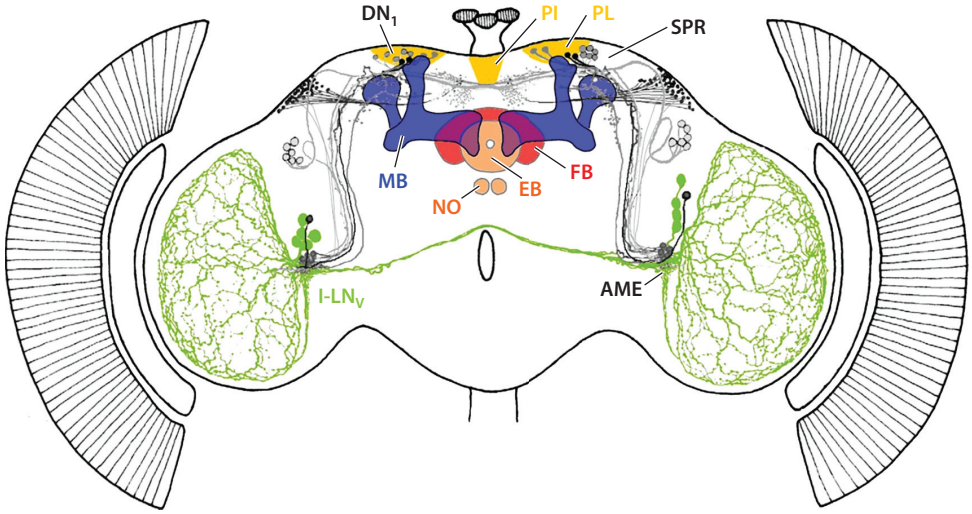

Figure 3

Location of the putative sleep-wake centers and the circadian clock network in the brain of *Drosophila melanogaster*. The protocerebral bridge of the central complex is not shown. The l-LN_v are part of the cicrcadian clock network. Other clock neurons and their arborization are depicted in gray. They arborize in the AME and in the SPR close to the putative sleep-wake centers. Abbreviations: AME, accessory medulla; DN_1, dorsal clock neurons 1; EB, ellipsoid body; FB, fan-shaped body; l-LN_v, large ventrolateral neurons; MB, mushroom body; NO, noduli; PI, pars intercerebralis; PL, pars lateralis; SPR, superior protocerebrum.

(66, 100). The PI and PL followed (47), succeeded by the simple idea that the PDF-expressing l-LN_v clock neurons alone are wake-promoting components of the *Drosophila* sleep circuit (25, 38, 94, 121). Finally, the CX became a prime candidate for housing the fruit fly sleep center (83, 140).

A few pathways connecting the clock neurons with the sleep-wake centers have been unraveled: a pathway from the dorsal clock neurons (the DN_1) to the PI and the CX (20, 21), a pathway from the lateral clock neurons (the s-LN_v) to the CX via leucokinin-expressing interneurons (22), and a pathway to the PL via AstA-expressing neurons (23). In the following sections, I discuss in detail the different putative sleep-wake centers.

Mushroom Bodies

The insect MBs are well known for their function in olfactory associative learning (57). In brief, MB dendrites in the calyces receive odor information from the antennae, and the vertical and medial lobes obtain information about the valence of these odors (good or bad) via dopaminergic and octopaminergic neurons, respectively (5, 14, 29, 112). Further input to all lobes comes from cholinergic neurons (148), and further input to the medial lobes comes from serotonergic/GABAergic neurons (56, 144). The MBs process this information and signal to the mushroom body output neurons (MBONs). The MBONs in turn project into specific areas in the dorsal brain (superior protocerebrum) to mediate adequate behavior (appetitive or aversive olfactory learning) (93). There seems to be a segregation into MBONs that are responsible for appetitive responses, such as the cholinergic or GABAergic responses, and MBONs that mediate aversive responses, such as the glutamatergic responses (6). Similarly, there might be specific dopaminergic neurons that mediate positive and negative valences of odors.

Although olfactory associative learning is the most important function of the MBs, it is not their only role. As early as 1960 and 1965, Huber (64, 65) noticed that blocking the MB pathway leads to elevated locomotor activity in crickets and grasshoppers (Orthoptera). A similarly higher activity level was observed in male fruit flies after ablating the MBs with hydroxy urea (61, 88). This finding suggests that the MBs usually send inhibitory signals to the locomotor centers. Joiner et al. (66) and Pitman et al. (100) demonstrated that fruit flies without functional MBs not only are more active but also sleep less than control flies. The latter studies also suggested that MBs are not entirely sleep promoting, but that they contain both sleep- and wake-promoting parts. Later work using restricted drivers to activate subpopulations of dopaminergic neurons (124) or to activate and silence MBONs (6) showed that the same dopaminergic and MBONs mediating negative odor valences and avoidance behavior also promoted wakefulness. Similarly, the neurons mediating positive odor valences and appetitive responses were sleep promoting.

The MB neurons targeted by the serotonergic/GABAergic neurons are wake promoting and inhibited by GABA, serotonin, or both. Thus, activation of these serotonergic neurons is strongly sleep promoting (56). This finding supports data showing that the loss of d5-HT1 serotonin receptors in the MBs made fruit flies sleep less (153). In contrast to the evident MB-mediated influences of dopamine and serotonin on sleep, octopamine seems not to affect arousal via the MBs (32). Whether ACh promotes wakefulness via the MBs or via other neuropils also remains unclear. Cholinergic neurons are widely distributed in the brain and innervate all neuropils (52, 150). ACh receptors are present in the MBs (148), and recent results show that the MB neurons themselves are cholinergic (8).

Pars Intercerebralis and Pars Lateralis

The PI and PL, with their connections to the corpora cardiaca/allata are the insect equivalents of the hypothalamus-pituitary system. Both PI and PL comprise many neuropeptidergic cells. The sleep- and wake-promoting effects of the neuropeptides EGFR, SIF-amide, DH44, and DILPs have been discussed above (20, 32, 47, 96). All these neuropeptides are expressed in different PI neurons. The PL contains other neuropeptidergic cells, such as AstA and leucokinin neurons, that seem involved in postprandial sleep (23, 90). This hypothesis gets support from a study showing that the DN_1 clock neurons located in this area affect the timing of the fruit fly siesta (53). Furthermore, two cell cycle regulators expressed in approximately 14 PL neurons were independently identified in genetic screens for short-sleeping mutants, though the mechanisms of this sleep control are not yet clear (1). The PI and PL belong to the most highly connected region within the fruit fly brain, and their primary role is most probably not to control sleep-wake behavior per se, but to integrate information about metabolic needs, stressful situations, and circadian time.

Large Ventrolateral Neurons

The l-LN_v are conspicuous clock neurons with wide arborizations in the optic lobe (**Figure 3**). They do not project into the superior protocerebrum as other clock neurons do, but they signal to most clock neurons via PDF released into the AME (42, 59, 110). A peculiarity of the l-LN_v is that they seem to show asynchronous circadian oscillations under constant conditions; they are, however, strongly responsive to light, mostly through the blue-light photopigment cryptochrome (45, 46, 121) and partly via the eyes (59, 89, 110).

The idea that the l-LN_v control fruit fly arousal and sleep was put forward in 2008 after the discovery that electrically hyperexciting them increases activity at night and disrupts nocturnal

sleep (121). Conversely, downregulating GABA receptors in the l-LN$_v$ either delays sleep onset if ionotropic RDL (Resistance to dieldrin) GABA$_A$ receptors are knocked down (25, 94) or disrupts sleep maintenance if metabotropic GABA$_B$ receptors are knocked down (51). This finding suggests that the l-LN$_v$ are wake-promoting neurons that need to be silenced via GABA at night to enable sleep. The sleep-promoting effects of GABA via the RDL GABA$_A$ receptors are mediated by WAKE, a molecule that acts as an accessory protein for RDL, upregulating expression levels and promoting membrane targeting (84). A homolog of WAKE is expressed in mouse SCN cells that receive light input and do not cycle under constant conditions, similar to the *Drosophila* l-LN$_v$.

The l-LN$_v$ receive GABAergic input but are also innervated by dopaminergic, octopaminergic, serotonergic, cholinergic, and histaminergic neurons (2, 25, 51, 77, 89, 94, 110, 153), suggesting that they are sleep-wake centers similar to the MBs, PI, and PL. Nevertheless, it is not clear whether these neuromodulators affect sleep via the l-LN$_v$. At least for dopamine, this is not the case: Although application of dopamine to isolated brains increases cAMP levels in the l-LN$_v$ (118) and appears to control sleep via the D1-like dopamine receptor, DopR1 (80), ablation of the l-LN$_v$ does not eliminate the strong arousal effects of dopamine (140). Altogether, this finding suggests that the l-LN$_v$ mediate light signals and arousal of the circadian clock network (117) rather than control sleep.

Central Complex

The CX, which consists of the protocerebral bridge, the upper and lower divisions of the central body, and the noduli, is a higher coordination center in the insect brain responsible for motor control and orientation (97, 143). The fan-shaped body (FB) of fruit flies corresponds to the upper division of the central body and coordinates motor actions (128, 151). The ellipsoid body (EB) corresponds to the lower division of the central body and is responsible for place memory (92) and startle responses to mechanical stimulations (80).

The first report of the sleep-promoting role of the FB appeared in 2011: Daytime sleep increased dramatically after depolarizing FB neurons that innervate the dorsal FB (39). Dopaminergic neurons from specific dopaminergic cell clusters inhibit these FB neurons and provoke wakefulness (**Figure 3**) (83, 140). These inhibitory dopamine responses are mediated by D1-like dopamine receptors that increase cAMP levels upon activation (83, 98, 140). Pimentel et al. (98) propose a detailed mechanism of dopamine action that relies on a sophisticated regulation of K^+ channels in the FB neurons and explains the counterintuitive inhibition of the FB neurons by D1-like dopamine receptors: In response to dopamine, the voltage-independent, leak current K^+ channel Sandman is upregulated and translocated to the plasma membrane. Consequently, the FB neurons switch to long-lasting hyperpolarization (OFF state), which keeps the fruit flies awake. The Rho-GTPase-activating protein Crossveinless-c locks FB neurons in the OFF state (36) until unexplained mechanisms flip the neurons back to the ON state. During the ON state, Sandman is internalized, the voltage-gated K^+ channels *Shaker* and *Shab* overtake, and the fruit flies can sleep (98).

In summary, sleep appears to be regulated by different parts of the *D. melanogaster* brain (**Figure 3**). This is perhaps not astonishing given the complexity of sleep. Nonetheless, in consideration of the connections between these brain parts, many of the discussed sleep pathways converge on the FB: The MBONs of the MB are connected to the FB via their terminals in defined regions of the superior protocerebrum that also comprise dendrites of FB neurons (5), SIF-amide neurons of the PI send fibers into the FB (96), and neurons in the PL carrying the leucokinin receptor project into the FB (22, 90). Taken together, the FB of the CX might be a master regulator in a hierarchical system that controls sleep and wakefulness. Recent data even

suggest that the CX plays a prominent role in sleep homeostasis (39, 40, 85): Upon waking, ring neurons of the EB increase their cytosolic Ca^{2+} levels, NMDA receptor expression, and structural markers of synaptic strength, and finally switch from spiking to burst-firing modes (85). This generates a sleep drive that is reduced only by subsequent sleep. The EB is connected to the FB (97) and thus can signal to it, again underlining the superior role the FB has in sleep regulation.

THE BIOLOGICAL FUNCTION OF SLEEP

The just mentioned results on the EB neurons support the synaptic homeostasis hypothesis, first proposed in 2003 by Tononi & Cirelli (137). It states that wakefulness progressively increases synaptic strength throughout the brain, because the awake brain learns and adapts to the ever-changing environment through synaptic potentiation. Overall, synaptic potentiation increases the energy consumption of the brain while decreasing the signal-to-noise ratio between essential and nonessential synaptic connections (i.e., essential and nonessential learning). The human brain uses as much as 25% of the whole-body glucose consumption, despite accounting for only 2% of body mass (125), and synaptic activity accounts for most of the brain's energy use. During sleep, the nonessential synaptic connections are scaled-down (renormalized), overall energy consumption is reduced, and new learning can take place. In other words, sleep is necessary for synaptic homeostasis. It is the price we pay for plasticity (i.e., learning).

The synaptic homeostasis hypothesis is supported by many observations of sleep in mammals and fruit flies (reviewed in 138, 139), but I mention only those of fruit flies: Overall levels of synaptic proteins increase after wake and decrease after sleep (50). Synapse size and number increase after only a few hours of wakefulness in the MBs and other brain structures and decrease only after the fruit flies are allowed to sleep (17). A richer wake experience results in larger synaptic growth and a greater sleep need (17, 38, 49). Sleep facilitates memory by blocking forgetting (16). Sleep deprivation disrupts short-term memory (49, 82, 115). Several short-sleeping mutants have impaired short-term memory (15).

Nevertheless, not all short-sleeping fruit flies have impaired memory and others are resistant to sleep loss, as measured by low sleep rebound and the ability to form short-term memories after sleep deprivation (reviewed in 36). Fruit flies in which sleep loss is provoked by octopamine, stress, and starvation do not show impaired memory either. If the synaptic homeostasis hypothesis holds true, either these fruit flies must sleep much deeper during their short sleep or they should not greatly increase overall synaptic strength during wakefulness. Future research has to show which of these two possibilities is true for fruit.

The brain needs a lot of energy even during sleep, because protein synthesis and repair processes are high (9). Because no food is taken up and heart rate and blood pressure are decreased, special physiological adjustments have to be taken to supply the brain with sugar. In humans, gluconeogenesis is highly upregulated in the liver so that the brain is continuously supplied with energy. In insects, not much is known about the measures taken to ensure sugar supply for the brain; a recent study in honey bees described a special ventilation mechanism in the abdominal dorsal air sacs that compensates for the absent ventilation movements during sleep and supplies the heart with oxygen so that it can effectively beat and transport nutrients to the brain (71). It will be interesting to test whether such mechanisms exist in other insects.

OUTLOOK

Although much work has been done and a large number of contributions have been made, sleep remains a fascinating state that is not completely understood. Further studies in the fruit fly have

the potential to unravel the precise brain areas involved in sleep control as well as the molecular mechanisms of sleep need. Studies in other insects, which have a rich behavioral repertoire, will help resolve the physiological mechanisms of sleep. It will also be most interesting to know the depth at which migratory locusts (*Locusta migratoria*) or monarch butterflies (*Danaus plexippus*) sleep and what happens to their brain during migration. If researchers involved in the sleep field focus on the inherent strength of insect systems, not only will their results be of interest to entomologists, but they may also be relevant to a greater audience.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

I dedicate this review to Irene Tobler in recognition of her pioneering work on animal sleep including insects.

I thank the DFG for continuous support of my work. During the writing of this review, I was supported by the grants Fo207/14-2 and SFB 1047 "Insect Timing." I thank Irene Tobler for her enthusiasm about sleep in insects that raised my interest; Thomas Riemensperger, Thomas Röder, Ricarda Scheiner, Monika Stengl, and Christian Wegener for discussions about sleep, feeding, and memory; Amita Sehgal for communicating unpublished results; Guntram Erbe, Ulrike Hausl-Hofstätter, and Paul Westrich for providing pictures of sleeping hymenopterans; Irina Wenzel for her help getting copyright permissions from publishers; and Marta Beauchamp, Monika Stengl, and Christian Wegener for critical comments on the manuscript. I apologize to all authors whose work I could not mention because of space constraints.

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