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Understanding the neurogenetics of sleep: progress from *Drosophila*

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Abstract

Most behaviors manifest themselves through interactions with environments. Sleep, however, is characterized by immobility and reduced responsiveness. Although nearly all animals sleep, the purpose of sleep remains an enduring puzzle. *Drosophila melanogaster* exhibits all the behavioral characteristics of mammalian sleep, enabling the use of powerful genetic approaches to dissect conserved fundamental neurogenetic aspects of sleep. *Drosophila* studies over the past four years have identified novel genes and pathways modulating sleep, such as *Shaker* and *sleepless*, and candidate brain regions known to function in circadian regulation and learning and memory. Advances in systems genetics coupled with the ability to target specific brain regions enable the characterization of transcriptional networks and neural circuits contributing to phenotypic variation in sleep.

Sleep: a classic enigma

Sleep is a unique behavior in that it renders individuals unable to interact with their environment. When asleep, individuals cannot forage for food, mate or defend against predation. Common sleep characteristics include daily extended periods of quiescence in which awareness of the external environment is suspended [1,2]. Sleep deprivation is balanced by subsequent attempts to regain lost sleep, a phenomenon known as sleep homeostasis [1,2]. These characteristics of sleep present a conundrum: one would expect selection against such a vulnerable behavioral state. However, sleep persists in virtually every animal species studied [1]. Sleep is crucial for survival, because classic experiments in rats [3] and recent experiments in flies [4,5] have demonstrated that long-term sleep deprivation results in death. Sleep research in humans has been hampered by the difficulty in controlling genetic background and environment. The observation that *Drosophila* exhibits

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the behavioral characteristics of mammalian sleep [6,7] enables the use of this powerful genetic model organism to advance our understanding of sleep because both the genetic background and the environment can be controlled precisely. In contrast to mammalian systems, populations of genetically identical flies can be generated rapidly, the creation of transgenic flies is fairly simple and the replication of experimental data is easily achievable. Furthermore, behavioral sleep parameters commonly measured in human studies can be readily computed in flies (Figure 1). Here, we discuss how recent *Drosophila* studies support hypotheses suggesting that sleep has a role in maintaining synaptic homeostasis and consolidating memory, how large-scale genetic screens and systems genetics techniques identify novel genes and natural allelic variants associated with sleep, and how spatially and temporally controlled gene expression reveal neural circuitry regulating sleep.

What is the purpose of sleep?

Many theories have been proposed for the function of sleep, including conservation of energy by reduced caloric expenditure [8,9], restoration of brain glycogen [10] and maintenance of synaptic homeostasis [11]. Some theories are based on the relationship of sleep patterns to physiology and have also been studied in *Drosophila* (Box 1). An additional intriguing hypothesis suggests that sleep is required for normal learning and memory [12]. Recent sleep research in *Drosophila* has focused on the potential relationship between sleep and learning and memory using the well-known courtship learning paradigm [13]. In this paradigm, a virgin male is 'trained' by a mated female, who rejects him. This rejection results in suppression of subsequent courtship behavior when he is introduced to a new 'tester' virgin female [14]. Interestingly, conditioned males sleep longer, even when compared with unconditioned males subjected to mechanical sleep deprivation [13]. When socially trained males are sleep deprived at different time points after their rejection by the female, the memory of their social experience can be obliterated. However, loss of memory only occurs during a crucial period, when flies are deprived of sleep within the first eight hours of the night [13]. If males are given 24 h of *ad libitum* sleep before sleep deprivation, they retain normal memory [13]. Furthermore, a known olfactory learning and memory mutant, *amnesiac*, also shows abnormal sleep [15]. Flies with fragmented sleep patterns are learning-impaired compared with controls having more consolidated sleep, even though both groups had the same amount of sleep in a 24-h period, an effect mediated by the dopamine DA1 receptor [16]. Although these findings demonstrate pleiotropic effects of single genes on both sleep and learning and memory, they do not provide evidence for causal relationships.

The notion that learning and memory consolidation is related to sleep can be extended to an effect of any waking experience on sleep patterns. Merely exposing a fly to other flies increases daytime sleep in a dose-dependent manner, implying that the intensity of waking experience alters sleep need [13]. This sleep increase requires sensory input from visual and olfactory systems. Sex effects also have an important role. Female–female pairs have activity patterns similar to single females. But male–female pairs have radically different activity patterns largely driven by the circadian rhythm of the male [17]. Alterations in sleep driven by waking experience mediated through social interactions are affected by mutations in short- and long-term memory candidate genes, such as *schnurri*, *Arleekin* and *elf-5c*, and

are dependent on dopaminergic transmission and cyclic adenosine monophosphate (cAMP) signalling [13].

An analysis of gene expression across fly, rodent and avian species supports the idea that waking experience necessitates sleep. This has led to the hypothesis that sleep is linked to synaptic homeostasis; if waking experiences strengthen synapses via long-term potentiation [11], sleep might counteract this effect to preserve metabolic resources [18]. Bushey and colleagues have tested this hypothesis through studies on *Fmr1*, a gene homologous to the human *FMRI* gene implicated in Fragile X syndrome. Sleep decreases with increasing *Fmr1* expression independent of waking activity [19]. Mutations in *Fmr1* are accompanied by changes in the structure of the mushroom bodies, central integrative structures of neuropils associated with learning and memory. Absence of the *Fmr1* transcript leads to increased branching of mushroom body synapses, whereas overexpression reduces synaptic complexity [20]. The correlation between *Fmr1* expression and sleep, however, is not simple because there is no homeostatic sleep response in either the absence or overexpression *Fmr1* message [19]. Although definitive proof of the synaptic homeostasis hypothesis requires the ability to monitor synaptic modeling in real-time, additional recent studies have made progress towards demonstrating the validity of this hypothesis. Gilestro and colleagues measured synaptic protein levels after exposing flies to different sleep and waking protocols [21]. Whether spontaneously awake or sleep deprived, levels of the synapse-associated proteins Bruchpilot, Discs-large, cysteine string protein, synapsin and syntaxin increase relative to spontaneous sleep or recovery sleep after sleep deprivation [21]. Confocal microscopy reveals increased Bruchpilot expression in the antennal lobes, mushroom body β lobes, ellipsoid body of the central complex and entire central brain in sleep-deprived flies relative to spontaneously sleeping flies [21]. A second study, which further investigated the effects of social enrichment on daytime sleep mentioned earlier, demonstrates that flies with mutations in *rutabaga*, *period* and *blistered* do not show experience-dependent sleep increases [22]. Expression of these genes in the circadian clock ventral lateral neurons rescues the mutant phenotype [22]. Synapse number in these clock neurons increases after social experience and decreases after sleep, supporting the notion that the purpose of sleep is to downscale synapses that are active during waking [22]. How synaptic downscaling during sleep can be reconciled with potentiation of learning and memory, processes that depend on synaptic strengthening, remains to be clarified. One can speculate that learning-related synaptic strengthening during sleep might be localized to distinct neural circuits, such as specific connections within the mushroom bodies, amidst more widespread synaptic downscaling.

The genetic underpinnings of sleep: from single genes to networks

Initial sleep genetic studies focused on the identification of mutations in single genes that alter sleep patterns. In one of the first large-scale forward screens, Cirelli and colleagues characterized sleep in 9000 fly lines – 6000 bearing ethylmethane sulphonate (EMS)-induced mutations on the X chromosome and 3000 bearing randomly inserted *P*-transposable element mutations. This study identified 15 lines that slept less than two standard deviations from the overall population mean [23]. The serendipitous observation that one of the EMS fly lines had a leg-shaking phenotype seen in previous X-chromosome

mutants enabled mapping of the EMS mutation to the gene *Shaker*, which encodes the α subunit of a potassium ion channel [23]. Intriguingly, a second large-scale screen of *P*-transposable element insertions revealed that *sleepless*, a mutation in a glycosylphosphatidylinositol-anchored membrane protein that resulted in very short-sleeping flies, impacts protein levels of *Shaker* [24]. Strong loss-of-function alleles of *Hyperkinetic*, which encodes the β -modulatory subunit of the *Shaker* potassium channel, have reduced sleep and a defect in short-term place preference memory, but a weak hypomorphic allele has no effect on either behavior [25].

Single gene mutations with large effects on sleep duration occur in *Drosophila*, but they are not common. Less than one percent of the lines screened in two mutagenesis studies had effects on sleep duration greater than two standard deviations [23,26]; however, many mutations had smaller effects on sleep, consistent with the results of *P*-element screens for other complex behavioral traits [27]. This suggests that the genetic architecture of sleep is determined by many genes, an idea long advanced by mammalian sleep researchers [28]. Indeed, contrasting whole genome transcript profiles between sleep and waking in the heads of standard laboratory strains reveals large numbers of differentially expressed genes [29]. Expression levels of most of these genes increase during waking [29,30]. Two independent studies show that gene products associated with lipid biosynthesis are upregulated during sleep [29,30]. In addition, three separate experiments identified increases in immune response transcripts during mechanically stimulated sleep deprivation [29–31]; however, many of these transcripts are also observed during mechanical stimulation at a time when the flies would normally be awake, suggesting that mechanical stimulation was the source of the immune response, not sleep loss [30].

EMS and *P*-element screens are efficient at identifying candidate genes involved in sleep, from genes of major effect down to the limit of detectable changes in phenotype. However, these allelic constructs do not occur naturally. Thus, EMS and *P*-element studies cannot reveal how variation for sleep might be maintained in natural populations. Many of the genes identified via EMS or *P*-element screens could in fact be invariant owing to intense selection pressure for a specific allele. Invariant alleles will neither explain sleep disorders in humans nor explain why sleep is conserved across taxa. Methodologies that take advantage of variation in natural populations, such as quantitative trait locus mapping, genome-wide association studies and systems genetics approaches, are designed to identify these naturally occurring alleles, with the assumption that alleles which are variable in flies will have some parallels in mammals. Screens of natural populations will not detect all genes that affect sleep, however, because they are dependent upon the allelic differences in the populations being studied. Thus, both types of studies are crucial to understanding sleep. EMS and *P*-element screens answer the question, 'what genes are involved in sleep?' Natural population-based approaches answer the question, 'what alleles maintain genetic variation for sleep?' Thus far, *Shaker* is the only gene found via a large-scale mutant screen which also exhibits transcriptional variation in natural populations [32].

When comparing different whole genome expression studies, there are few genes that are common. Such discrepancies can reflect the use of different tissue preparations (such as fly heads versus brains) [33], but disparate genetic backgrounds and rearing conditions might be

more crucial factors [34]. There are two possible strategies to ameliorate these problems. One is for all investigators to use a standardized genetic background and maintain the flies under identical conditions. The other, more practical solution is to embrace the genetic background diversity and use it to one's advantage. In a recent study, we used this approach by capitalizing on naturally occurring phenotypic variation in sleep phenotypes and whole-fly transcript profiles among a population of 40 wild-derived inbred lines [32,35] (Figure 2). Instead of looking at gene expression within one background or averaging across genotypes, this study regressed variation in sleep phenotypes on variation in transcript abundance to identify quantitative trait transcripts (QTTs) (Figure 2a,b). We then asked to what extent QTTs could be clustered such that each transcript within a cluster is more strongly correlated with members of the same cluster than with members of other clusters (Figure 2c). This unbiased clustering algorithm identified groups of statistically inter-correlated genes with biologically relevant functions in broad Gene Ontology categories that include metabolism and transcription and protein binding, localization and transport [32]. This systems genetic approach makes it possible to uncover highly inter-connected genes most crucial to natural genetic variation in sleep (Figure 2d). Future studies are necessary to assess the extent to which human orthologues of *Drosophila* genes within these networks are associated with natural variation in human sleep phenotypes and identify potential orthologous candidate genes that harbor risk alleles for sleep disorders, such as insomnia and narcolepsy.

Neural circuitry for initiation and maintenance of sleep

The relative ease with which transgenes can be introduced in flies makes *Drosophila* an exceptionally powerful organism to functionally dissect and identify neural circuits with an important role in sleep. The versatile *GAL4-UAS* binary expression system (see Glossary) provides a convenient genetic tool to manipulate spatially controlled transgene expression. Neural circuits associated with sleep were identified by screening *GAL4* drivers that activate a dominant-negative temperature-sensitive allele of a *shibire* transgene controlled by a *UAS* promoter [36]. *shibire* encodes a dynamin necessary for synaptic vesicle recycling; expression of the temperature-sensitive dominant-negative mutant at the non-permissive temperature results in neuronal silencing. These experiments implicate the mushroom bodies in sleep duration [36]. Further evidence for the regulation of sleep duration by mushroom bodies comes from experiments targeting expression of the constitutively active catalytic subunit of protein kinase A, an enzyme activated by the sleep-relevant cAMP pathway [37], to different brain regions [38]. In both studies, green-fluorescent-protein expression pinpoints the mushroom bodies as important regions in the brain impacting sleep [36,38]. However, one study concluded that the mushroom bodies promote sleep [36], whereas the second hypothesized that sleep could be promoted but also inhibited by different subsets of mushroom body neurons [38]. The implication of the mushroom bodies in sleep regulation in two independent studies provides additional support for the hypothesis that sleep affects learning and memory, because the mushroom bodies are the primary center for learning and memory in the fly. However, sleep is reduced, but not absent, when mushroom bodies are chemically ablated by hydroxyurea [36,38]. These results suggest that additional brain regions are involved in the regulation of sleep [38]. This idea is supported by studies on the

effects of octopamine, the insect equivalent of noradrenaline in vertebrates, on sleep. Increases in octopamine, whether by oral administration or genetic manipulation of the octopamine biosynthesis pathway, reduce sleep [39]. Although octopamine can affect PKA signalling, its effect on promoting wakefulness does not seem to occur in the mushroom bodies [39]. The site of action of octopamine remains to be determined.

Furthermore, the pars intercerebralis region of the *Drosophila* brain has been implicated in sleep regulation. In a 2007 study, inhibition of the epidermal-growth-factor-receptor pathway in the mushroom bodies had no effect on sleep; however, when this pathway was silenced in the pars intercerebralis sleep was reduced [40], providing the first evidence that neural circuits other than the mushroom bodies can regulate sleep. How these circuits are functionally integrated remains to be determined.

To what extent are neural circuits that regulate circadian rhythms integrated with sleep?

The biological clock in flies includes a group of large ventral–lateral neurons (l-vLNs) and a cluster of small ventral–lateral neurons (s-vLNs) [41] (Figure 3a). Transgene expression can be targeted specifically to these neurons with a *Gal4* driver construct that includes the promoter region of the *pigment dispersing factor* (*pdf*) gene, the expression of which is largely restricted to l-vLNs and s-vLNs in the fly brain [41]. When vLNs are excited using either a voltage-gated potassium channel or a bacterial voltage-gated sodium channel transgene, flies exhibit reduced sleep independent of waking activity [42–44]; sleep latency also increases [42,43]. Excitation of the vLNs is thought to be mediated by light [42–45]. In a series of elegant experiments, Shang and colleagues used constructs that enabled activation of a voltage-gated sodium channel transgene in subsets of circadian neurons and showed that daily activity is positively correlated with the number of hyper-excited neurons [44]. Thus, genetic manipulations that mimic the neuronal excitation of circadian neurons can impact sleep in a dose-dependent manner.

Additional experiments targeting *pdf* itself suggest that it is also required in the regulation of sleep. Reduced or null expression of PDF receptors increases sleep, as do mutations in *pdf* [42,46]. PDF mutants also have a dampened response to arousing stimuli [46]. Thus, PDF, a known output protein of the molecular circadian clock, is also required for normal sleep.

A mutation in *Resistant to dieldrin* (*Rdl*), which encodes a γ -aminobutyric acid (GABA)-A receptor, increased sleep by decreasing sleep latency, demonstrating a conserved role for GABA-A receptors between flies and mammals in promoting sleep [47]. Interestingly, Chung and colleagues also found *Rdl* in an RNAi screen for regulators of PDF neurons [46]. Parisky and colleagues have demonstrated that *Rdl* protein expression in the brain is largely restricted to large and the small vLNs [42]. Overexpression of *Rdl* in these neurons increases sleep and reduces sleep latency [42]. These experiments identify the l-vLNs and s-vLNs as a physical link, and *pdf* and *Rdl* as molecular links between the circadian clock and sleep in the *Drosophila* brain (Figure 2b). It should be noted that, in all of these experiments, sleep patterns are altered but not eliminated. As in mammalian systems, this poses the continuing dilemma of to what extent a single focal group of neurons or a few neurons regulate sleep

or, as is more probable, whether sleep regulation is broadly dispersed across many brain regions.

Extending the results from flies to mammals

Candidate genes for sleep in *Drosophila* are likely to have relevance to humans. Two studies to date have made a direct connection between flies and mammals. In the first study, a putative biomarker for sleep need was identified using microarray analyses of fly heads after flies were subjected to several different sleep deprivation protocols [48]. Transcript levels of amylase increase consistently with increasing levels of sleep deprivation. Similarly, salivary amylase activity and mRNA levels increase in humans after 28 h of waking [48]. Measures of biomarkers such as amylase could be used to assess sleep deprivation levels in humans before the behavioral and cognitive manifestations (difficulty making decisions and decreased alertness and sleep latency) of sleep deprivation occur.

A second study tested the mouse *Kcna2* gene product, the closest mammalian homologue of *Drosophila Shaker*. Sleep was recorded 17 days after birth in *Kcna2* knockout mice. Homozygous *Kcna2* knockouts have 23% less non-rapid eye movement (NREM) sleep than heterozygotes and wild types and died of seizures ~28 days after birth [49]. They did not show changes in rapid eye movement (REM) sleep and had no apparent circadian defects. Wild-type and heterozygous mice did not have substantively different sleep, even at later ages [49]. Thus, initial studies are promising and show that fly candidate genes for sleep duration are likely to have orthologues with conserved functions in mice and humans.

There are, however, some caveats when extrapolating findings from sleep in *Drosophila* to mammals. Whereas sleep in flies has all the behavioral characteristics of mammalian sleep, the widely used infrared monitoring system cannot distinguish between sleeping animals and those that are simply inactive, resulting in inaccuracies in sleep measures [50]. Furthermore, flies do not seem to have the complex electrophysiologically defined sleep architecture present in mammals [51]; thus, studies in *Drosophila* will not be able to distinguish the purpose of REM as opposed to NREM sleep. Despite these limitations, studies in *Drosophila* sleep can be instrumental, like studies of circadian rhythms in which the fundamental concept of a molecular feedback loop enabled the discovery of mammalian core clock genes [52].

Current and future trends

The pioneering observation that *Drosophila* sleep mimics mammalian sleep [6,7] has enormous potential for sleep research. Although some major advances have been made in identifying genes that control sleep in mammalian systems, the speed with which mutations in flies can be tested for their effect on sleep is far greater than for any other organism. During the past four years, 15 500 fly lines bearing EMS or *P*-element insertional mutations have been assayed for sleep phenotypes [23,24,26], novel genetic pathways with effects on sleep patterns have been identified and transcriptional networks that encompass hundreds of genes have been correlated with sleep parameters [32]. The assumption that sleep candidate genes are conserved across taxa has been demonstrated by the *Shaker* homologue in mice [49]. However, these mutagenesis studies also reveal that large-effect mutations are not

common; in fact, distributions of mutational effects suggest that many genes can influence sleep [23,24,26,53]. Furthermore, large effects can be masked by modifiers [23], implying that epistatic interactions might be common. Finally, the environmental impact on genes controlling the neural regulation of sleep remains to be characterized.

Drosophila provides an ideal model system for elucidating how whole genome transcriptional regulation enables specific neural circuits to control sleep. This problem can be studied at two levels of complexity, the genome and neural connectivity, which ultimately have to be combined. Although single gene studies have made substantial advances, it is becoming increasingly clear that sleep is a complex quantitative trait that emerges from interactive networks of pleiotropic genes. Recent advances in systems genetics now enable the characterization of transcriptional networks that contribute to phenotypic variation in sleep and robustness in the face of genotype-by-environment interactions and gene-by-gene interactions.

Because fly brain regions bear little resemblance to mammalian brain structures, evolutionary conservation of sleep implies conservation of cellular and molecular interactions co-opted by different neural circuits during the evolution of nervous systems across taxa [1]. The relative simplicity of the *Drosophila* brain provides a favorable scenario for elucidating the neural circuits that mediate sleep. The ability to control spatial and temporal regulation of transgenes in *Drosophila* has been instrumental in identifying major behavioral regions of the fly brain in sleep regulation. Although the mushroom bodies, pars intercerebralis and ventral lateral neurons have been implicated in sleep, the ablation of specific brain regions does not completely abolish sleep behavior [36,38,43,44,46], suggesting that, like mammals, neural regulation of sleep in *Drosophila* is diffuse and controlled by multiple neural circuits. Elucidating temporal causality in sleep regulation between different neural circuits will require new technology that enables comprehensive real-time monitoring of neural activity in living flies with single-cell resolution. Also, activity in distinct neural pathways, including synaptic remodeling, must be correlated with transitions between wakefulness and sleep. Such studies could definitively confirm or refute the synaptic homeostasis hypothesis. If in the future such applications become possible and if technology becomes available to simultaneously survey whole genome transcriptional regulation in real time *in vivo*, it will become possible to obtain a truly comprehensive understanding of sleep. Whereas realization of this goal might seem to be far in the future, the extension of findings from flies to humans using current technology will prove important for identifying therapeutic targets for sleep disorders and for understanding the impact of sleep in human life.

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Glossary

<i>GAL4-UAS</i> binary expression system	a transgenic construct that places expression of the yeast transcription factor GAL4 under a cell-specific promoter which in turn results in cell-specific activation of transgenes behind GAL4-driven upstream activating sequence (UAS) promoter sites.
Non-rapid eye movement (NREM) sleep	a phase of sleep characterized by low neuronal activity and metabolic rate. It is divided into four stages. Stage I represents the transition from waking to sleep. Stage II can have two EEG patterns known as sleep spindles (a sinusoidal wave) and K complexes (a high voltage biphasic wave). Stage III marks the beginning of slow-wave sleep, when the EEG has slow, high-voltage waves. Stage IV has greatly increased slow-wave activity. Stages III and IV are the deepest stages of sleep.
Rapid eye movement (REM) sleep	a phase of sleep, characterized by rapid eye movements, electroencephalogram waves that resemble the waking state and the occurrence of dreams.

Box 1**Physiology and sleep**

Drosophila sleep patterns change with changes in physiology. Like sleep patterns in humans, sleep becomes more fragmented as flies age [55]. Young flies have long, uninterrupted sleep bouts that occur mostly at night. Sleep in older flies becomes more evenly distributed across the 24-h day. Correlated with changes in sleep patterns during aging are changes in the strength of circadian rhythms, which suggests that the circadian clock exerts some influence over sleep consolidation. The rate of this decline in the strength of circadian activity with age can be altered by changes in temperature and exposure to oxidative stress through administration of paraquat [55]. Flies can therefore effectively model the effects of aging on sleep.

Flies also serve as models for studies on different states of arousal. Anesthetized flies are physiologically similar to sleeping flies. Local field potentials recorded under isoflurane show a similar decay in brain activity to that seen in sleeping flies [51,56]. Furthermore, ablating dopaminergic neurons or overexpressing dopamine alters the sensitivity of flies to anesthesia, demonstrating that similar neurotransmitter pathways are affected in anesthesia and sleep [51]. However, optic lobe potentials are reduced in amplitude with increasing doses of anesthetic [51,56], whereas no such phenomenon occurs in sleep [56]. Thus, flies can be employed to study different states of arousal.

Finally, metabolic state interacts with sleep. Glycogen stores and sleep duration are positively correlated in male flies, as is average sleep-bout length [53]. The number of sleep bouts, however, is negatively correlated with glycogen [53]. In contrast to males, glycogen stores are not correlated with any measure of sleep in females. Here, triglyceride stores are positively correlated with average sleep-bout length and negatively correlated with sleep-bout number [53]. These results show that metabolic state might influence sleep in a sex-specific manner. A relationship between metabolic state and sleep is also prominent in people because human studies show a correlation between reduced sleep, obesity and diabetes [57]. Thus, studies in flies can inform studies on the relationship between aging, metabolism and sleep in humans.

TRENDS in Genetics

Figure 1.

Sleep parameters in humans and flies. **(a)** The traces are representative of electroencephalograms (EEGs) depicting each sleep stage (see Glossary for additional details). The graph depicts a typical night of sleep, which progresses from waking to the four stages of NREM sleep and then to REM sleep in a cycle that lasts ~90 min. Sleep at the beginning of the night is predominantly NREM and sleep at the end of the night is predominantly REM sleep. Part (a) courtesy of C. Cirelli. **(b)** The graph shows an example of activity patterns in a *w¹¹¹⁸; Canton-S* female fly. Activity counts averaged into 5-min bins are plotted for a two-day period. From these activity patterns, sleep parameters can be calculated as shown in the diagram below the graph. The fly in this example has two day-sleep bouts on the first day and four on the second day (i.e. three day-sleep bouts on average). Similarly, the fly has eight night-sleep bouts on the first day and six on the second, which averages to seven night-sleep bouts. Adding up the minutes spent in each sleep bout gives the total day and night sleep duration. Average bout length is computed by dividing sleep duration by the number of sleep bouts. The sleep latency of the fly is the time in minutes from the beginning of the night until the start of the first sleep bout, as shown in the figure for the first night period. Waking activity is the total number of activity counts divided by the minutes spent awake.

Figure 3.

Circadian clock neurons that might control sleep in *Drosophila*. **(a)** The image shows a frontal view of the brain showing large and small ventral lateral neurons (l-vLN and s-vLN, respectively) immunolabeled with pigment dispersing factor (PDF). Part (a) adapted, with permission, from Ref. [54]. **(b)** Light activates l-vLNs, which release pigment-dispersing factor onto s-vLNs that in turn project to other clock neurons and other brain regions such as the ellipsoid bodies (EB) that are involved in control of locomotion. Both l-vLNs and s-vLNs express GABA_A receptors, which might enable sleep-promoting GABAergic neurons to suppress wakefulness. Part (b) adapted, with permission, from Ref. [42]. Abbreviations: OL, optic lobes.