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Phylogenetically distant animals sleep: why do sleep researchers care?

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Abstract
Philosophers examining mechanistic explanations in biology have identified heuristic strategies scientists use in discovering mechanisms. This paper examines the heuristic strategy of investigating phylogenetically distant model organisms, using research on sleep in fruit flies as an example. At the time sleep was discovered in flies in 2000 next to nothing was known about mechanisms regulating sleep in flies and what they could reveal about those in us. One relatively straightforward line of research focused on homologous genes in flies and humans, using those in flies to understand what roles their homologs played in controlling sleep in us. But other research focused on a higher level of organization—the neural networks involved in homeostatic and circadian control of sleep. This raises a puzzle—given that fly and vertebrate brains are organized very differently, how could sleep regulation in flies serve as an informative model of vertebrate sleep? I argue that the basic design of mechanisms such as those regulating sleep can be conserved even as the composition of the mechanism changes and that researchers can hope to use the designs deciphered in flies as heuristic models for understanding sleep in humans.

Keywords Sleep · Model organisms · Fruit flies · Mechanism discovery · Discovery heuristics

Introduction

The last two decades have witnessed a succession of findings that animals that seem further and further phylogenetically distant from humans nonetheless engage in the common human activity of sleeping. For example, recently researchers have reported

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sleep in two additional species of *Cnidaria*—the upside down jellyfish, *Cassiopea* (Nath et al. 2017) and *Hydra vulgaris* (Kanaya et al. 2020). What is the point of this research? Is finding sleep in species phylogenetically far removed from us just a curiosity? Although the finding that organisms of an unsuspected species sleep is what attracts popular excitement, it is the research that follows that should be of interest to philosophers of science. The finding of sleep in the fruit fly (*Drosophila melanogaster*, hereafter *fly*) in 2000 has spawned a research effort aimed at generating new insights into the mechanisms responsible for the phenomenon of sleep.[1] While they are investigating the mechanisms in flies, researchers' implicit and sometimes explicit hope is that their findings about mechanisms might extend to humans. My goal in this paper is to analyze how researchers established the fly as an organism in which to study sleep, the investigations they have conducted on flies, and how the results of these investigations can be employed as heuristic models for understanding sleep in humans.

Both vertebrate and invertebrate sleep researchers aim not just to elucidate the phenomenon of sleep but to discover the mechanisms controlling it. When asleep an organism relies on the same physiological processes as it does when awake, but deploys them differently (downregulating some, maintaining or even upregulating others). Accordingly, sleep results from the operation of control mechanisms—mechanisms that operate on other mechanisms to modify their operation (Winning and Bechtel 2018). Discovering the mechanisms controlling sleep in humans has proven quite challenging. Progress has mostly occurred in describing the phenomenon of control of sleep. Observations of sleep in us and other mammals reveals both that sleep is typically associated with a particular time of day (night in us, day in mice) and that when sleep is missed, organisms exhibit a rebound effect, sleeping longer in subsequent sleep periods. Drawing on these two features of the phenomenon, Borbély (1982) advanced a two process model of sleep, with a homeostatic process S operating like an hour-glass timer measuring the buildup of sleep pressure over the time that an organism is awake and a circadian process C determining periods of the day when the organism is permitted to sleep (Daan et al. 1984; Borbély and Achermann 1999). Another phenomenal feature of sleep is the abrupt transition between waking and sleeping. This has lead Saper et al. (2010) to hypothesize that a switch mechanism is involved.

Experimental investigations on mammals have not revealed the mechanisms responsible for the two processes or the sleep switch. In part the challenge stems from the fact that in the mammalian brain there is not one localized area—a sleep

[1] A similar research effort was initiated in the nematode worm following Raizen et al.'s (2008) demonstration that although worms did not exhibit daily episodes of sleep, during development they enter a quiescent state (during which they are less responsive to stimulation) known as *lethargus* prior to each of its four molts. Following on the findings of this initial paper, researchers have shown that lethargus exhibits many of the same features as daily sleep in other organisms (e.g., they sleep longer in the next bout if prevented from sleeping during one bout) and employ many of the same mechanisms. One of the genes required for sleep timing, *lin-42*, is an ortholog of the circadian gene *per*. Yet more recently, worm researchers have demonstrated sleep states in worms during sickness or injury or when satiated. This research has revealed the existence of different mechanisms responsible for sleep in different circumstances, a finding that suggests for examining the differences in sleep mechanisms involved in nighttime sleep and daytime naps in humans. For discussion, see Bechtel and Bich (2023).

center—regulating sleep. The lack of localized control of sleep is not all that surprising. As a phenomenon that appears to be manifest in all animals with neurons,[2] control mechanisms that govern the transition into sleep states likely evolved very early in phylogeny, perhaps with the evolution of neurons as specialized cell types. Even if in the first animals that slept there was one or a small number of specialized mechanisms that controlled sleep, over the course of evolution additional neural processes, largely devoted to other activities, were likely integrated into the mechanisms controlling sleep so as to restrict sleep to the times when it was least disruptive or when the benefits it provided were most needed. Jacob (1977) characterized evolution as a history of tinkering and the product of such tinkering is not likely to conform to our ideal of a well-organized, modularized system.

Since its inception in the 19th century, biologists have adopted the practice of investigating organisms other than humans (or other target organism) to seek insights into biological mechanisms. In this process, they implicitly treat both the phenomena studied and the mechanisms advanced as general even as they recognize differences manifest in the organisms studied. As Ankeny and Leonelli (2020) describe, in the late 20th century some organisms, such as the fly, were designated model organisms. In part this reflected the development over that century of stocks of specially bred laboratory strains of the species and investigatory tools, especially at the molecular level, for studying them. It also reflects the organization of communities of scientists who promoted research on specific organisms, sharing knowledge about both research strategies and results. Although the number is still small, some philosophers have analyzed the use of model organisms in biology. Some have investigated how researchers select model organisms to use in particular investigations (Burian 1992, 1993; Schaffner 1998a, b, 2001). Other philosophers have engaged the question of when inferences from model organisms to target organisms (often, but not always, humans) are justified (Levy and Currie 2014; Steel 2007; Weber 2005). Among the most compelling factors for extrapolating findings from model to target species is shared ancestry, which is most often established in terms of genes. Homologous genes are those descended from a common ancestral gene and the assumption is that if the genes coding for the proteins constituting the mechanism are homologous, the proteins in both organisms carry out versions of the same operation. It is important to note that homologous genes are not identical and that the operations they perform will not be identical. Even when they are related by descent, researchers expect differences between the mechanisms in different model organisms. One might better regard inferences from mechanisms in model organisms as heuristic inferences: researchers may take the mechanism in the model organism as a basis for reasoning about the mechanism in the target organism, but also recognize that major changes may have occurred over the course of evolution.

One reason researchers were motivated to investigate sleep in flies is that the fly is a well-developed model organism with rich research tools. Yet, in 2000 virtually nothing was known about the mechanisms underlying fly sleep. What motivated researchers to devote the resources needed to investigate sleep mechanisms in them?

[2] There are a few examples of animals lacking neurons such as *Trichoplax adherens*; sleep has not yet been identified in them.

In picking model organisms to investigate, one strategy is to study species that are phylogenetically close to the target, as those would be ones most likely to generate extrapolatable results. This explains the use of mice and rats to study a wide range of phenomena, including sleep. But flies are phylogenetically distant. A very different strategy is to investigate the simplest organism in which one can identify the phenomenon of interest. Weber (2005, p. 176) considers simplicity: "It might be suggested that the best-suited organisms for studying a particular process will be the simplest ones that actually contain this process." However, he sets it aside, offering three considerations for doing so: simplicity is not spelled out, there are always other simple organisms that could have been chosen, and there are many cases in which researchers choose a less simple organism. Weber doesn't explain why researchers do, on some occasions, emphasize simplicity. When the goal is to understand mechanisms, investigating simpler mechanisms may reveal the basic processes and how they are organized to enable the mechanism to exhibit a class of phenomena. I will refer to this as the basic design of the mechanism. Given a process of evolutionary tinkering, one might expect it to still be operative, but obscured, in later evolved versions of the mechanism. Discovering the basic design of the sleep mechanism is a major motivation of researchers studying sleep in flies.

As Weber notes, the notion of simplicity needs to be spelled out.[3] No organisms currently alive are truly simple. In the lineages of all extant organisms each predecessor was able to carry out the activities needed to live and was able to compensate for the challenging conditions it confronted. This requires a complex system. Moreover, evolution has continued to tinker with every organism descended from a common ancestor. But the tinkering does not alter all organisms equally, leaving some in a relatively simpler state. Two measures of simplicity that are highly relevant to investigating mechanisms are less redundancy and fewer layers of regulative control (Greenspan 2007). Reduced genetic redundancy increases the likelihood that a mutation or other genetic manipulation will result in an interpretable result (Bell et al. 2009). Since control mechanisms often serve to protect the mechanism from interruptions, in organisms with fewer controls researchers are able more easily to manipulate the mechanism in ways that reveal its working parts and how they contribute to the phenomenon. This is the major consideration motivating investigating phenomena in the simplest organisms that exhibit them.

My focus will be on how biologists have gone about pursuing flies as models in which to study sleep with the hope of utilizing the knowledge to better understand human sleep. As a first step, I discuss in Sect. 2 the challenge researchers faced in establishing that flies sleep and how this was overcome. In Sect. 3 I examine the initial investigations into sleep in flies, showing that it followed a strategy discussed by Weber—focusing on homologous genes implicated in sleep and using the fly as a system in which to identify the operations performed by the proteins coded for by those genes. While this strategy has been productive, it is limited in providing understanding of what lower-level components do in one or more mechanisms that

[3] Historically, simplicity was invoked at the level of the genome—model organisms were taken to have smaller genomes. This has not turned out to be the case (Ankeny and Leonelli 2020). My focus is on mechanisms, not genes.

regulate sleep, not how the overall mechanism works. To acquire that understanding, researchers moved up a level of organization to the neurons in which the proteins are expressed and the ways these neurons interact in the control of sleep. To understand how researchers directed their inquiry to this higher-level of organization and identified circuits in the fly brain that regulate sleep, I discuss research on homeostatic sleep regulation in Sect. 4 and circadian sleep regulation in Sect. 5. While this is still work in progress, so far research at this level of organization has been successful in producing suggestive sketches of the mechanisms that control sleep in flies.

Moving up to a higher level in a model organism generates a new challenge—the fly brain is organized very differently than that of vertebrates. How can researchers apply what they learn about the neural mechanisms that regulate sleep in the fly to inform research on vertebrates? I address this issue in Sect. 6, arguing that it requires that we expand the notion of conservation to include conservation of mechanism activities and organization—the mechanism design. The organized set of operations that regulate sleep in the fly can be conserved even as the components that perform specific operations are modified. Different components can perform similar operations and be organized in similar ways into larger mechanisms. If that is what has happened in the case of control of sleep, then flies can be a model for the design of the mechanisms controlling sleep in mammals despite the large-scale differences in brain organization.

Redefining the criteria for sleep to include flies

Sometimes the way a phenomenon is characterized is an obstacle to identifying it in other species. In the 20th century sleep researchers supplanted our folk ways of identifying whether a person is asleep (whether a person is quiescent, laying down or letting their head drop, and somewhat difficult to arouse) with an electrophysiological measure using e*lectroencephalography* (EEG). Shortly after Berger (1929) had discovered that he could detect and record electrical oscillations from electrodes placed on a person's skull and that these rhythms corresponded to the behavioral states of the person,[4] other researchers measured brain rhythms during sleep and identified waves with higher amplitude but slower frequency than those reported by Berger. Loomis et al. (1937) identified a progression through five stages from rhythms while quietly resting to those of deep sleep.[5]

[4] Berger (1929) found that when participants were quiet and kept their eyes closed, they exhibited high amplitude oscillations of approximately 10 Hz (which he referred to as *alpha* waves). When participants opened their eyes or responded to stimuli, the frequency would increase to between 20 and 30 Hz and the amplitude would decrease (Berger 1930, called these *beta* waves). Subsequent research revealed yet higher frequency, lower amplitude *gamma* oscillations when participants are engaged in cognitively demanding tasks.

[5] Among the more surprising findings when researchers began to study sleep with EEG was that periods of slow-wave oscillations were periodically interrupted by periods of low-amplitude, high-frequency oscillations that resembled patterns found when individuals were awake. These states were correlated with eye movements that did not involve other activities of the waking state and were identified as periods of rapid-eye movement (REM) sleep (Aserinsky and Kleitman 1953).

EEG provided a non-intrusive way of quantifying sleep in humans. It could also be applied to other mammals. Borbély himself conducted his research on sleep homeostasis on laboratory rats and showed that process S remained even when circadian rhythms were eliminated by lesions to the master circadian clock in the suprachiasmatic nucleus (Tobler et al. 1983). Also working on rats, Rechtschaffen et al. (1983) established that sleep deprivation could be fatal. But EEG cannot be applied to aquatic animals and those without a neocortex. Thus, to the degree it became the measure of sleep, it prevented the study of sleep in organisms in which one cannot record EEG.

Some researchers resisted the restrictions reliance on EEG as the measure of sleep imposed and investigated the behavioral manifestations of sleep in a wide variety of animals. Tobler (1983) studied sleep in cockroaches. Campbell and Tobler (1984) compared the amount and timing of sleep in over 150 animal species and reported on sleep behavior in invertebrates such as insects as well as in fish, amphibians, reptiles, birds, and mammals. To conduct such a comparison, they drew upon the much earlier research of Piéron (1913) and employed four behavioral measures of sleep:

> (1) the assumption of a stereotypic or species-specific posture,
> (2) the maintenance of behavioral quiescence,
> (3) an elevation of arousal threshold which may be reflected in the intensity of an arousing stimulus and/or the frequency, latency or duration of an arousal response, and.
> (4) state reversibility with stimulation (pp. 269–272).

While Tobler and a few others studied sleep behavior in insects, their efforts did not lead to widespread efforts to identify mechanisms controlling insect sleep. This changed rapidly after Hendricks et al. (2000) and Shaw et al. (2000) established that fruit flies, a model organism for which extensive research tools had been developed, satisfied the behavioral criteria of sleep. To make their case that flies sleep, both groups of researchers maintained flies in sealed tubes with food at one end and tracked their movements visually or with an automated system that recorded whenever a fly interrupted an infrared beam directed through the midpoint of the tube. Their results showed that the flies spent periods immobile at a location away from the food. Periodically the researchers would try to arouse the flies by vibrating the tubes. Finding that when inactivity exceeded five minutes, a stronger stimulation was required to arouse the flies, the researchers adopted 5 min of inactivity as the criterion for sleep. Based on it, they determined that flies typically sleep 7.5 h per day. Periods of complete immobility, where the only movements were those associated with respiration, were often short, with the longest lasting 26 min. Periods interrupted only by extensions or retractions of the proboscis or abdominal twitches lasted as long as 157 min.

In reporting their findings, neither group of researchers referred to the state as *sleep*. Hendricks et al. (2000) argued that fruit fly rest is a "sleep-like state" while Shaw et al. (2000) contended that the flies exhibited the "correlates of sleep and waking." This initial caution in equating the behaviors in flies with sleep has gradually been cast aside as researchers began calling the phenomenon "sleep." Today, as witnessed by recent review articles, it is common to refer to flies and other organisms

that satisfy the behavioral criteria, but for which EEG is not possible, as sleeping (Dissel 2020; Dubowy and Sehgal 2017; Keene and Duboue 2018; Miyazaki et al. 2017; Shafer and Keene 2021; Tomita et al. 2017).

Part of the reason for acceptance that flies sleep is that fly researchers did not simply show that flies satisfied the criteria laid out by Campbell and Taylor. They also demonstrated that flies exhibited other behaviors comparable to those exhibited by sleeping mammals. First, they exhibit a daily rhythm in sleep behavior. Flies are most active at dawn and dusk. In between, they exhibit sleep bouts, with sleep bouts during the night being more consolidated. Flies maintain the pattern of two periods of highest activity separated by periods of sleep bouts every 24 h even in constant darkness, indicating these events were controlled by each fly's circadian clock. Second, flies exhibit sleep rebound: if deprived of sleep for a night (e.g., by the researchers regularly tapping on their tubes), flies sleep three to seven times longer than usual the next night.[6] Third, Shaw et al. reported that aging flies slept less (a finding followed up on by Koh et al. 2006). Finally, Shaw et al. (see also Andretic et al. 2005) found that fly sleep is sensitive to some of the same drugs as affect sleep in humans: caffeine reduces sleep while antihistamines result in more sleep.[7] Research also revealed that in flies a variety of physiological and behavioral phenomena such as metabolic rate (Stahl et al. 2017), aggression (Kayser et al. 2015), and immune responses (Williams et al. 2007) are altered during sleep.

Once they had compelling reasons to adopt behavioral criteria for sleep, fly researchers could identify sleep without EEG. Nonetheless, they remained interested in the altered electrophysiological activity during sleep that EEG is used to measure in mammals. To pursue this interest, they turned to local field potentials—potentials reflecting electrophysiological activity in the surrounding region recorded from electrodes inserted into the brain. Using local field potentials, Nitz et al. (2002) demonstrated reduced neuronal activity during periods of sleep. This was later supported by studies using GCaMP to measure Ca^{2+} levels (Bushey et al. 2015). In addition, van Alphen et al. (2013) demonstrated varying levels of electrical activity during sleep bouts and showed that these levels correlate with behavioral responsiveness to stimuli. Faville et al. (2015) describe an automated means for measuring arousal threshold and showed that flies exhibited the highest thresholds early in the night and then cycle between lower and higher thresholds through the rest of the night. They also found that thresholds increased during sleep bouts, reaching a maximum after 30 min of inactivity. Using LFPs, Yap et al. (2017) found oscillations of 7–10 Hz in sleeping flies as flies are beginning to sleep, with reduced or desynchronized activity later in sleep bouts, suggesting the flies go through different sleep stages.[8] These

[6] Huber et al. (2004) established more precise quantitative relations between time awake and sleep recovery and determined that sleep recovery is less fragmented and exhibits higher arousal thresholds than baseline sleep. Further, they demonstrated that sleep deprivation affected vigilance and performance, measured in terms of activity in response to a stimulus.

[7] More recently, Keebaugh et al. (2017) showed that the effect of caffeine is indirect, mediated by changes in feeding behavior.

[8] More recently, van Alphen, Semenza Evan, Yap, van Swinderen, and Allada (2021) inferred that different stages of fly sleep serve different functions. They identified a period of deep sleep, measured by increased arousal thresholds and reduced neural activity, that was manifest in repeated extensions and

different lines of research have generated a rich characterization of the phenomenon of sleep in flies.

Using flies to investigate molecular components of the sleep mechanisms

Beyond characterizing the phenomenon of sleep, many investigators seek to understand the mechanisms that give rise to the phenomenon. As noted by many of the philosophers discussing model organism research, a major motivation of such research is that it enables the deployment of well-developed tools for investigating processes at the molecular level. Especially in cases in which it is difficult to investigate the role of specific molecules in the target organism but where homologs to the genes for those molecules are present in the model organism, researchers use the model organism to reveal what the molecules do. Much of the initial research on fly sleep followed such a path.

I begin with the classic strategy in genetic research of creating mutants that show deficits in a phenomenon of interest and inferring from the deficits how the unmutated gene/protein contributes to the phenomenon. For example, Cirelli et al. (2005) created a short-sleeping mutant fly, *minisleep*.[9] This mutant fly exhibits the same number of sleep episodes as wildtype flies, but of shorter duration, resulting in the fly sleeping only 4–5 h per day. A clue to the deficit was that these flies exhibited a transient leg shaking and wing scissoring when recovering from diethyl ether anesthesia. This pointed researchers to the *Shaker* locus, which had been identified much earlier and shown to code for an α-subunit of the voltage-dependent potassium channel protein involved in membrane repolarization and transmitter release (Jan et al. 1977). Noting evidence that potassium channels are involved in generating sleep rhythms in mammals, Cirelli et al. propose "It is possible that the *mns* mutation, by affecting an ion channel that controls membrane repolarization, may be close to the core cellular mechanisms of sleep" (p. 1090).

In subsequent research Bushey et al. (2007) extended this finding by showing that another mutant with a mutation at the *Shaker* locus, *Hyperkinetic*, also exhibited reduced sleep. This mutant has a mutation in a modulatory β-unit that interacts with the α-subunit of the protein. Koh et al. (2008) identified a further component of the mechanism with the generation of *sleepless* (*sss*), an extreme short-sleeping mutant. Dean et al. (2011) demonstrated that the SSS protein figures in the activation of Shaker. Subsequent research showed that SSS binds with part of the nicotinic (ionotropic) acetylcholine receptor (nAChR), directly inhibiting it (Wu et al. 2014).

retractions of their proboscis that was not being elicited by gustatory stimuli. Determining that flies prevented from extending and retracting their proboscis die and that the frequency of proboscis extension during sleep increased after injury, they treated it as serving to clear out molecules that accumulated in the nervous system during waking.

[9] Kume et al. (2005) created another short-sleeping fly they named *fumin* (Japanese for sleepless) that inactivated the dopamine transporter, revealing the role of dopamine in maintaining wakefulness. For a detailed review of the role different monoamines and neurotranmitters more generally play in regulating sleep in flies, see Ly et al. (2018).

The link to the acetylcholine receptor was further supported when Shi et al. (2014) identified a short-sleeping mutant *redeye* and demonstrated that the gene codes for the α subunit of the nAChR. (I discuss Shaker further in Sect. 4.)

Shaw et al. (2000) pursued a different strategy for identifying fly genes that affect sleep: looking for genes that are differentially expressed during periods of waking and sleeping. Among the genes known to be upregulated during sleep in rats, the researchers identified the fly homolog of Hsc70-3, the endoplasmic reticulum chaperone protein binding immunoglobin protein (BiP). BiP is the master regulator of the unfolded protein response pathway that, in response to stress signals from the endoplasmic reticulum, reduces protein synthesis, upregulates the production of chaperones to increase protein folding, or increases the degradation of misfolded proteins. Naidoo et al. (2007) found that the concentration of BiP protein doubled after three hours of sleep deprivation but gradually returned to baseline over 24 h when the flies are allowed to rest. Moreover, increased expression of BiP results in increased recovery sleep, while increased expression of a dominant negative mutant form reduces recovery sleep.

Further research on flies has revealed parts of the mechanism through which BiP affects sleep. BiP acts through the protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) pathway in which it phosphorylates PERK. The phosphate is then transferred to the α-subunit of eukaryotic initiation factor 2 (eiF2α). When eiF2α is phosphorylated, it forms a stable complex with the guanine nucleotide exchange factor eiF2B to halt synthesis of new proteins. Ly et al. (2020) demonstrated that phosphorylated eiF2α levels rose during waking hours and especially with sleep deprivation and that two different pharmacological impairments of PERK blocked both the inhibition of protein synthesis and sleep. They obtained similar effects with genetic knockdown of *PERK*. With overexpression of *PERK*, sleep increased. Ly et al. showed that restricting these perturbations to PDF expressing neurons known to figure in circadian control of sleep (see below) resulted in either decrease of PDF (with overexpression of *PERK*) or increase (with knockdown of *PERK*).

The research just described, as well as many other research endeavors reviewed by Ly et al. (2018), used flies to develop clues as to how genes/proteins known to be homologues of those found in mammals contribute to sleep. By focusing on effects in mutants in which the gene in flies is mutated or in flies when a given gene is expressed at higher than normal levels, researchers developed hypotheses about what operations the associated proteins perform that contribute to sleep. These results provide leads for mammalian researchers to follow in determining the roles the conserved proteins play in more complex systems. While these operations contribute to sleep, they are in an important respect identified at too low a level to generate understanding of the mechanism responsible for sleep. For that, researchers turned to the neurons and neural circuits that regulate sleep. In the next two sections I discuss how they have done so in hopes of understanding the two processes Borbély identified—homeostatic and circadian regulation of sleep.

Using flies to identify neural structures involved in homeostatic control of sleep

As discussed above, one of the challenges in understanding homeostatic control of sleep in mammals is that there is not a localized responsible mechanism—neurons that affect sleep seem to be distributed widely throughout the spinal cord and brain. This is true in the fruit fly as well, but since it has a considerably smaller brain (approximately 100,000 neurons), the number of neurons forming these circuits is smaller, making it easier to identify both individual populations of neurons involved in sleep and the circuits they form. Moreover, as is generally true in invertebrates, there is less variability between individual organisms, making it possible to identify the same neurons and their patterns of connectivity in different flies. Following the trail of research pioneered in the nematode *C. elegans* (White et al. 1986), researchers are developing maps (connectomes) identifying each neuron and how it connects to others. Based on serial electron microcopy of a single female fly, Scheffer et al. (2020) have recently published a connectome map of one hemisphere. Drawing upon the standardization in the fly brain, researchers have succeeded in identifying a relatively small collection of neurons[10] that figure in homeostatic control of sleep. Most of these are found in two neuropils (dense networks of dendrites and axons, with cell bodies residing outside): the mushroom bodies (MBs) and the central complex (CC). Within the CC, sleep regulating neurons have been found in two structures: the fan-shaped body (FB) and the ellipsoid body (EB) (Fig. 1).

The mushroom bodies were the locus of the first success in finding neural mechanisms regulating sleep in the fly brain. Two papers that were published back-to-back in *Nature* in 2006 initiated the investigation. Pitman et al. (2006) showed that application of a temperature-sensitive synaptic blocker operating through the MBs resulted in reduced sleep. Joiner et al. (2006) localized molecules that figure in sleep to the MBs. Following up on studies showing an inverse relation between activation of CREB via cAMP and protein kinase A (PKA) and sleep (Hendricks et al. 2001), Joiner et al. tested different activators of PKA and found that two different drivers known to localize to the MB altered sleep in different directions—one increasing sleep and one decreasing it.

Subsequent research has focused on the structure of the MBs, in which approximately 2000 Kenyon cells (KCs), located in the Calx (the part of the MB that resembles a mushroom), send axons to the 34 Mushroom Body Output Neurons (MBONs), located in the three vertical and medial lobes shown in blue in Fig. 1. Axons from MBONs in turn project to areas in the superior protocerebrum in which neurons elicit different behaviors (Helfrich-Förster 2018). Joiner et al. localized the molecular drivers of the waking and sleeping genes they identified in different regions in the lobes, suggesting that some MBONs are sleep-promoting and others wake-promoting. Independent of its role in sleep, the architecture of the MBs has been extensively investigated to understand the roles it plays in processing and assigning valence to odors. This provided a foundation for Aso et al. (2014) to identify five glutamatergic

[10] Glial cells figure centrally in many activities attributed to neurons. Recent research has found that this is also true of sleep (Blum et al. 2021; Jepson 2021).

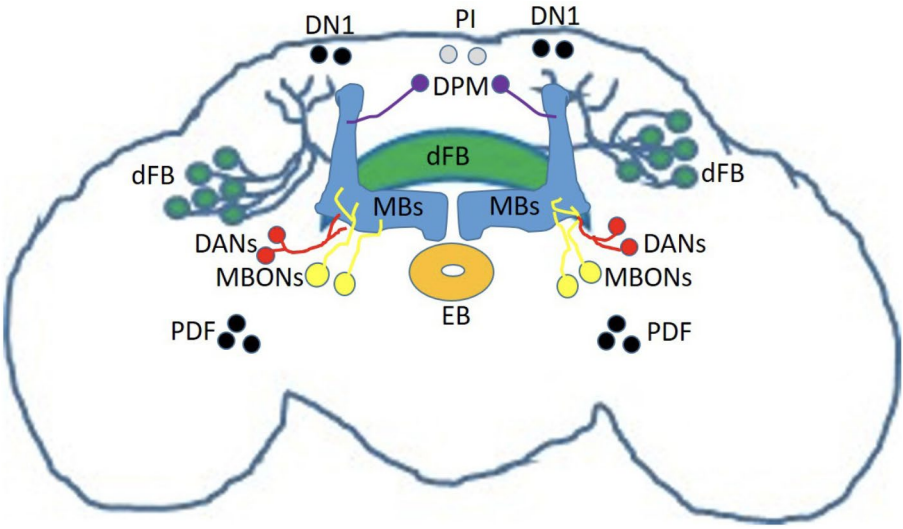

Fig. 1 Regions in the fruit fly brain viewed as involved in sleep regulation. The mushroom bodies (MBs) receive inputs from dopamine neurons (DANs) and dual paired medial (DPM) neurons and output to the mushroom bodies output neurons (MBONs). The dorsal fan-shaped body (dFB) and the ellipsoid body (EB) belong to the central complex. The DN1 and PDF neurons, shown in black, are part of the circadian clock mechanism discussed in Sect. 5. From Dissel (2020)

MBONs that support sleep and seven serotonergic or GABAergic neurons that support waking. Sitaraman et al. (2015) traced the inputs to these MBONs to specific KCs. These microcircuits helped explain sleep phenomena such as sleep rebound: the sleep promoting circuit was more active after sleep deprivation and without it, sleep deprivation does not result in rebound sleep. The same research group worked out some details of these circuits, showing how dopamine (released into the MBs by PAM neurons) activates the wake promoting circuits (Sitaraman, Aso, Rubin, & NitabaSitaraman et al. 2015a, b). Meanwhile, Haynes et al. (2015) established that GABA released from DPM neurons suppresses wake promoting neurons. These findings pointed to the MB circuits as figuring in registering the need for and promoting sleep. Aso et al. (2014) also traced the outputs from sleep neurons in the MBs, finding that in one case both sleep promoting and wake promoting neurons targeted the same neuropils, the superior medial protocerebrum (SMP) and the crepine (CRE). They inferred that through the inputs to these neuropils, the MBONs regulated the transition between sleep and waking "by providing opposing inputs to shared downstream targets" (p. 19). These neuropils contain dendrites of neurons whose cell bodies are in the CC.[11]

In recent years research on the CC, a neuropil situated, as the name suggests, in the center of the fly brain, has provided insights into mechanisms responsible for Borbély's process S and the sleep switch. The CC consists of four substructures, of which

[11] Kirszenblat and van Swinderen (2019) raise doubts about whether MBs are involved in sleep control, suggesting rather that they are involved in the decision of whether to move or not given an odor signal and are switched off during sleep.

two—the ellipsoid body (EB) and the fan-shaped body (FB)—have been implicated in regulating sleep (Fig. 2). Like the MBs, the neuropils in the CC are involved in other information processing; in particular, neurons in the EB are implicated in place memory and responses to mechanical stimulation while those in the dorsal FB (dFB) are involved in coordinating motor activity (Helfrich-Förster 2018).[12]

Research is pointing to neurons in the EB as registering sleep pressure. Liu et al. (2016) identified a subset of EB neurons, originally designated R2 but more recently categorized as R5 (Omoto et al. 2017), that register sleep drive through an increase in firing that is proportional to the time since sleep. The investigators determined that the increased activity results from an increase in the number of active zones in pre-synaptic neurons and NMDA receptors and calcium activity in post synaptic neurons. These decline after sleep, resulting in reduced firing of R5 neurons. Dendrites of R5 neurons are found adjacent to the CRE and SMP neurons to which MBONs project, suggesting that they receive information about sleep pressure in part from the MBs. They also receive inputs from helicon cells, discussed below.

Turning to the dFB, Donlea et al. (2011) identified a population of neurons (subsequently designated ExFl2) that undergo a sharp transition from promoting wakefulness to promoting sleep. Recognizing how this population satisfied the specifications

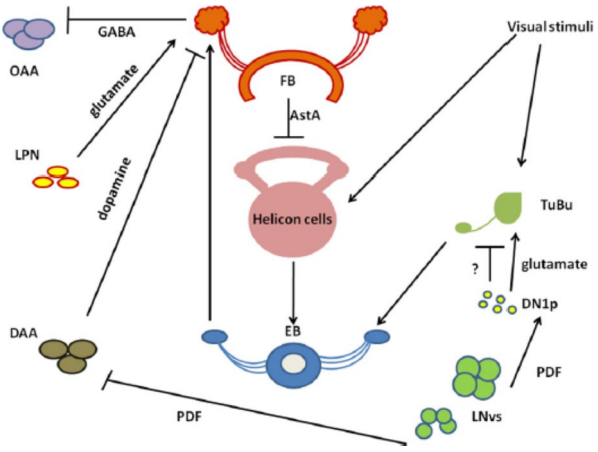

Fig. 2 Circuit involving two key regions in the central complex that regulate sleep, the fan-shaped body (FB) and the ellipsoid body (EB). The FB both generate sleep by sending inhibitory GABA outputs to output arousal neurons (OAA) and participate in a circuit in which they inhibit helicon cells that normally excite neurons in EB, which in turn activate neurons in the FB. Other areas acting on the FB are the dopamine arousal neurons (DAA) and the lateral posterior neurons (LPN). On the right-hand side, the role of light is shown as both directly affecting helicon cells and, via tubercular bulbar cells (TuBu), EB neurons. The inhibitory effects on DAA neurons of the ventral lateral neurons (LNvs; part of the circadian network) in releasing pigment dispersing factor is also shown, as is the effect of LNvs on TuBu neurons, mediated by other circadian neurons—dorsal neurons posterior (DN1p). From (Mazzotta et al. 2020)

[12] Based on the fact that many of the genes expressed in the CC and vertebrate basal ganglia are homologs, Strausfeld and Hirth (2013) argue that the CC functions similarly to the basal ganglia in deciding which activities to pursue.

advanced by Saper et al. (2010), Pimentel et al. (2016) called it a sleep switch.[13] They drew upon the molecular research discussed in the previous section to explain how these neurons generated switch-like behavior as a result of the interaction of two potassium channels that oppose each other. One involves the Shaker protein—when in high concentrations, Shaker allows the neuron to repolarize rapidly after an action potential, thus increasing its firing rate. This is the ON state that promotes sleep. The OFF-state results from the relocation of another protein, Sandman, to the membrane where it closes a leak current K^+ channel, keeping K^+ inside and hyperpolarizing the cell. The switch is flipped back ON by moving Sandman away from the membrane, allowing Shaker to dominate.

One consideration that makes it plausible that the dFB neurons act as a switch is that they are positioned both to receive competing inputs that can lead to switching and to generate outputs that activate sleep behavior (Fig. 2). They receive input indicating the buildup of sleep pressure from the population of R5 neurons in the EB and other populations that register sleep pressure (SIF-amide neurons of the pars intercerebralis and leucokin-receptors neurons in the pars lateralis). In opposition to these, they receive input from several populations of dopaminergic neurons that upregulate Sandman, pushing the switch to the OFF position and maintaining a wake state.

ExFl2 neurons have been shown to send projections to several populations of neurons that regulate waking or sleeping behavior. Of particular note are a populations of neurons Donlea et al. (2018) identified and named helicon cells in reference to their shape (Fig. 2). Donlea et al. found this population by looking for receptors for the neuropeptide allatostatin-A (AstA), which they took to be released by dFB neurons. (As I discuss in Sect. 4, AstA may not actually be released by the dFB neurons but by nearby populations of neurons involved in circadian regulation.) Donlea et al. showed that helicon cells receive visual inputs and generate motor commands, and so are appropriate targets to be inhibited when the sleep switch is ON. When it is OFF, not only is locomotor behavior allowed but, as a result of projections to R5 neurons in the EB that Donlea et al. identified, sleep pressure accumulates. Assuming either AstA or some other signal is transmitted from ExFl2 neurons to helicon cells, the result is a loop in which, when the sleep switch is OFF, helicon cells enable locomotor activity and build up sleep pressure in R5 neurons. When that is sufficient to flip the switch to ON, helicon cells no longer enable activity, allowing sleep pressure registered in R5 neurons to drop.

Moving up from the level of genes and proteins to that of neurons, researchers have identified populations of neurons in the MBs and the CC in the fly brain that figure in control of sleep homeostasis. In both cases, they have begun to advance hypotheses as to how circuits composed of these neurons operate. Research on these mechanisms is still in an early stage, and the proposed mechanisms are likely to be revised and further elaborated as a result of future research. But the mechanism sketches developed

[13] Helfrich-Förster (2018, p. 78) referred to it as "a master regulator in a hierarchical system that controls sleep and wakefulness." For doubts about whether it represents a switch, see Kirszenblat and van Swinderen (2019), who propose that it may act to disrupt processing of sensory stimuli, which mimics a transition to sleep, Instead of a switch, they hypothesize "Maybe there is no single sleep switch, but rather a number of different brain areas that, when coordinated, guide waking behavior and when out of joint promote sleep."

so far provide models that can be used to guide research on mammals, including us, as they reveal a mechanism that is able to register sleep deficits and act on switches that change from promoting waking to enabling sleep. I return in Sect. 5 to the challenges in applying this model to brains that organized very differently than flies. First, though I consider how research on flies is providing an understanding of the second of Borbély's processes controlling sleep, circadian control.

Using flies to investigate the interaction of circadian rhythms and sleep

Circadian regulation of sleep limits the times of day during which specific organisms sleep. In the case of flies, this is both during the night and during what is referred to as the siesta between early morning and early evening. The core circadian mechanism in all eukaryotic organisms is an intracellular molecular mechanism that, in animals, also depends on coordination between neurons in the brain. Although researchers have discovered a great deal about both the intracellular molecular mechanism generating rhythms and the circuits coordinating rhythms in populations of neurons, research is only now revealing how these circadian rhythms modulate various behaviors (not just sleep, but feeding, temperature preference, and eclosion from pupae in the case of the fly).[14] Research on flies played a major role in the discovery of the core circadian mechanism and is currently playing an important role in elucidating the multicellular networks that figure in circadian control of sleep.

The intracellular circadian mechanism in all eukaryotic cells generates oscillation through a process in which the proteins synthesized by select genes feed back to inhibit the expression of these genes until the inhibitors degrade. In flies, two proteins, Cycle and Clock, act as transcription factors for the synthesis of Period and Timeless. Once they are expressed, Period and Timeless form a dimer, are transported back into the nucleus, and prevent Cycle and Clock from acting as transcription factors until they degrade (Fig. 3). These core operations are supplemented by others, including components of other feedback loops, that enable the concentrations of Clock, Period, and Timeless to oscillate with a regular period of approximately 24 h (Nitabach and Taghert 2008). As the period is not precisely but only approximately 24 h, the core mechanism must continually be entrained to the light-dark cycle in the fly's environment. Light can penetrate into the fly's brain and acts on a light sensitive protein, Cryptochrome, that modulates the feedback loops that constitute the clock mechanism.

Although the core circadian mechanism is intracellular, King and Sehgal (2020) identify approximately 150 neurons in the fly that are primarily responsible for circadian timekeeping and so constituting the central circadian clock. These form different populations labeled in Fig. 4.[15] Neurons in each population behave somewhat differ-

[14] On circadian regulation of different physiological and behavioral functions, see Katewa et al. (2016).

[15] The cell processes of all these neurons are found in the accessory medulla (AEM), a small neuropil at the base of the medulla (itself situated at the base of the second optic ganglion). The cell bodies are widely dispersed.

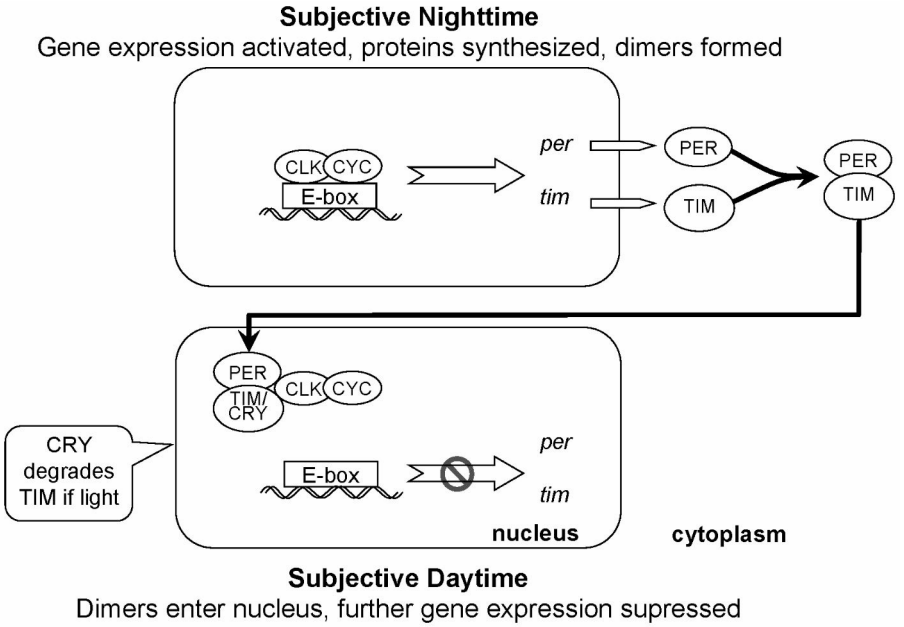

Fig. 3 Core circadian mechanism in flies in which the dimer of Period (PER) and Timeless (TIM) acts on the transcription factors Clock (CLK) and Cycle (CYC) to remove them from the site (E-box) where they promote Period and Timeless synthesis. Cryptochrome (CRY) registers light and causes the degradation of TIM

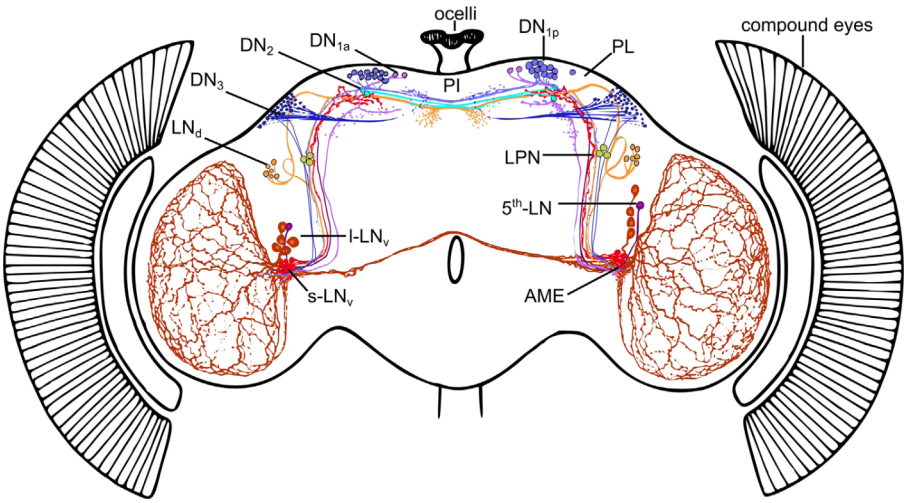

Fig. 4 Location of the populations of neurons constituting the central clock in the fly brain. From (Reinhard et al. 2022)

ently. They are interconnected, sometimes bidirectionally, resulting in what Top and Young (2018) refer to as the *circadian clock neuronal network* (subsequently, *clock network*).[16] A major role in coordinating neurons across the clock network is played by the neuropeptide pigment dispersing factor (PDF) that is released by just a few of the neurons in the network—four pairs of small ventral lateral neurons (sLNvs) and four pairs of large ventral lateral neurons (lLNvs).[17] Not only the LNvs but also other members of clock network—the DNs and the LNds—have receptors for PDF that enables them to align their oscillations with those of the PDF releasing neurons.

Although their molecular activity is coordinated, measurements of Ca^{2+} concentrations reveals that the LNvs (except the fifth sLNv) and the DN1p generate more action potentials in the morning than other times (and so are known as the *morning oscillator*) while the LNds and the fifth sLNv do so in the evening (constituting the *evening oscillator*) (Liang et al. 2016). The peaks of these two oscillators correspond to the two periods in which flies are most active, suggesting that the different populations within the clock network drive activity during these two periods. These peaks respond differently to light: the morning peak occurs earlier with earlier daylight and the evening peak later with later daylight, facilitating coordination with daylength as it varies over the course of the year.

Despite having developed a relatively detailed understanding of the core mechanism and the clock network, researchers are only beginning to figure out how this system regulates various behaviors. I focus on what researchers are learning about how the clock network regulates sleep. One strategy that has proven successful is to start with sleep inducing neurons and to determine which of them are affected by the circadian clock. Cavanaugh et al. (2016) identified a set of 201y-GAL4+neurons that project to the dFB in which manipulations promoted or inhibited sleep. They found that this effect was limited to particular times of day, having little effect during the morning or evening activity period. This time limitation, however, was not manifest in flies in which the central clock was disrupted, which the investigators took to establish that these neurons served to integrate outputs from the circadian clock with processes regulating sleep. Recently Andreani et al. (2022) have shown a circadian effect on the registration of sleep pressure in the EB. Focusing on sleep rebound, they demonstrated an effect of DN1p neurons in enhancing the morning rebound and LNds as suppressing the evening rebound, offering a "model of a circadian regulated homeostat that turns up late at night to sustain sleep and down late in the day to sustain wake" (p. 17).

A particularly promising line of research has focused on the three lateral posterior neurons (LPNs) in each hemisphere. The LPNs are located very near the dFB neurons which, as discussed earlier, Donlea took to be the sleep switch and to release the AstA that acted on helicon cells. Ni et al. (2019) determined that the actual source of AstA was the LPNs. The sleep switch neurons have a receptor for AstA, and the researchers

[16] Although the clock network is important for maintaining synchronized clock activity, cells throughout the fly maintain circadian oscillations: glial cells in the brain, retinal photoreceptor cells, the antennae and other sensory organs, the heart, the kidneys, the liver, the gonads and the cuticle (Helfrich-Förster 2017).

[17] The lLNvs, located adjacent to the AME, figure importantly in the light input pathway that entrains the fly clock to the light-dark cycle in its environment (Helfrich-Förster 2020).

proposed that these receptors are the site of a competitive interaction between AstA and dopamine, known to promote waking.

Based on their identification of multiple interactions between components of the circadian network and sleep regulating neurons, Reinhard et al. have sketched a network (Fig. 5). In developing this network, they differentiated the three LPNs and identified multiple targets to which each project. The ones that project to the dFB also project to the MBs as well as to a locus in the pars intercerebralis (PI). Chen et al. (2016) identified AstA responsive neurons in the PI as regulating feeding and metabolism during the morning and proposed that they acted to prepare the fly for an energy-saving state during the midday siesta. Reinhard determined that the third LPN rhythmically syntheses two other peptides, Allatostatin C (AstC)[18] and Diuretic Hormone 31 (DH31),[19] which act on other circadian populations (DNs and LNds), and, either via them or directly, affect activity in the PI. Through the different projections from the LPN to the PI, the LPN can exert multiple influences on feeding behavior and metabolism.

As with the mechanisms engaged in the homeostatic regulation of sleep itself, investigations of modes of interaction between the clock network and the components regulating homeostatic sleep are still in an early stage. Even so, the research is identifying populations of neurons that act differently in generating circadian rhythmicity. The network advanced by Reinhard et al. provides a foundation on which to understand how circadian rhythms act on, and are affected by, the neurons involved in homeostatic regulation of sleep. This research offers a heuristic model for research into how circadian mechanisms in humans and other mammals.

[18] (Zhang et al. 2021) demonstrate a role for AstC in rhythmically inhibiting reproduction.

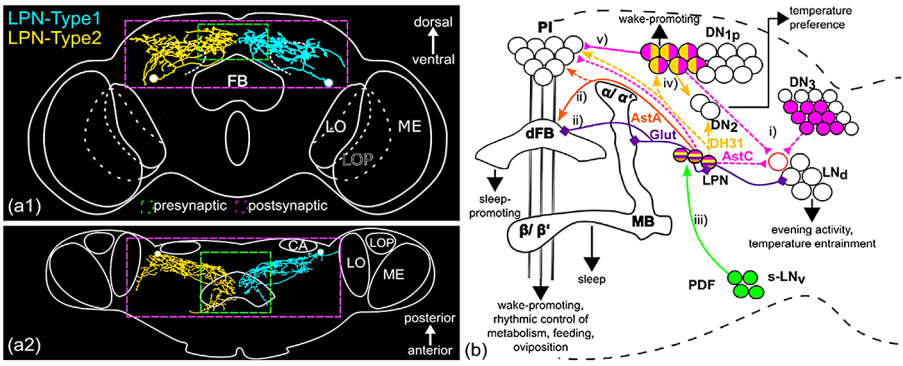

Fig. 5 Proposed network of neural populations engaged in circadian rhythms and sleep regulation. At the center of this proposal are three LPN neurons, which receive PDF input form sLNvs **(iii)**. Two LPNs send outputs, via glutamate and AstA, to neurons in the dFB, MBs, and the PI regulating sleep **(ii)**. The third LPN signals via DH31 to wake promoting DN1 neurons **(iv)** and via AstC to LNd, which also receive inputs from DN1 and DN3 neurons **(i)**. Via AstC, this neuron and DN1s act on neurons in the PI. From (Reinhard et al. 2022)

[19] DH31 is the homolog of the vertebrate wake-promoting neuropeptide calcitonin and is wake-promoting in flies (Kunst et al. 2014).

Using the fly to guide research on homeostatic and circadian mechanisms in mammals

In the previous two sections I illustrated how fly researchers are developing accounts of the mechanisms involved in homeostatic and circadian regulation of sleep in the fly with the aspiration that what they discover can be applied to target organisms such as us. In this section I focus on the challenge of making inferences about sleep mechanisms from this research. Ankeny and Leonelli (2020) treat model organisms as like other models in science and construe models as representations. Insofar as representations stand in for what they represent and are used to reason about them, this perspective is promising. But it is important to consider more specifically how model organisms serve as stand-ins. When the model organism is phylogenetically close to the target, researchers can hope that the entities in the model organism can stand in for the entities in the target and that researchers can then apply what they learn about the entities in the model to the target. For research on conserved genes, this account also works reasonably well even with phylogenetically distant model organisms—one can view the gene in the model organism as a stand in for its homologs in the target organism and project what is learned from the gene in the model onto the gene in the target. But, as I have discussed, much of the research on sleep in flies is at a level above that of genes and proteins, involving neural structures and circuits in the fly's brain. Here phylogenetic distance seems to undercut the ability of using the mechanisms in the fly as representations of those operative in us. The MBs, the dFB, and the components of the circadian network do not map in any straight-forward way onto structures in the vertebrate brain. Without these mappings, how can one understand the homeostatic and circadian sleep mechanisms in the fly as representing those mechanisms in us?

I have been emphasizing the attractiveness of investigating sleep mechanisms in the fly as due to the fact that the mechanisms in the fly are simpler. However, something can be simpler but yet totally different. If the simpler mechanism employs very different principles, there is little to be learned about the more complex mechanism from studying the simpler ones. For the relative simplicity of the model organism to be beneficial, the model organism must provide a simpler version of the mechanism found in the target. In what sense can one understand the sleep mechanisms in the fly to be simpler versions of the ones in us? To address this question, one needs to further address the use of models to represent targets. The motivation for looking to the fly as a model is that the mechanisms in it can be viewed as representing those in the common ancestor in which the basic design of the mechanism first developed. The goal of this representation is to reveal the basic principles employed in the mechanisms that produce the phenomena. The assumption guiding the research is that just as the mechanisms actually present in the fly resulted from evolutionary tinkering beginning with the ur-mechanism, so have the mechanisms found in us. If that is the case, then the ur-mechanisms, and by extension the ones in the fly, can be informative about the ones in us. What is derived from research on the fly is a particular generalization—one that captures the basic design principles realized in the ur-mechanism and its various descendants and that can be invoked to explain how all of the organisms produce the common phenomenon.

The basis for generalizing from model to target is that what is present in the model is conserved in the target. But when it is the design of the mechanism that is being projected from the model onto the target, the focus is not on the conservation of genes (although that may enter into the projection) or of conserved structural components. The parts of the fly and human brain do not map onto each other in any obvious way that allows researchers to view them as conserved. Rather, it is the design of the mechanism—the set of operations and how they are connected—that is conserved.

Viewing the design of the mechanism as conserved does not mean that it is simply retained. Evolutionary tinkering continues to modify the design over time. The process of tinkering can account for how one can have conservation of mechanism design without conservation of components. One form of tinkering involves replacing components with others that perform the same activity (plus perhaps others). There is good reason to think this has happened at the molecular level with the circadian clock. While some components of the fly clock are homologs of those in the mammalian clock, others are completely different proteins that perform much the same function (e.g., a form of Cryptochrome has replaced Timeless as the dimerization partner of Period and acts in inhibiting the transcription of itself and Period). Looking more broadly, the set of activities and organization in the circadian clocks in plants and fungi are remarkably similar to those in animals, suggesting that even at the molecular level, mechanism design can be conserved even as the components change.

Applying this perspective to the homeostatic and circadian mechanisms in flies and us, researchers do not need to be able to map components of the mechanism to use one as a model for the other. Rather, they can attempt to map basic design principles, elicited from the fly, to mammals. The assumption underlaying this endeavor is that the basic design has been conserved (and expanded upon). The findings discussed in the previous two sections provide some examples of designs that might be considered. The research on homeostatic regulation of sleep in the fly revealed a loop relating a switch involving neurons in the dFB that acted on a sensory-motor pathway passing through the helicon cells, which generated sleep pressure in neurons in the EBs that, in turn, sent inputs to the switch neurons. This design might be realized by neurons distributed in different parts of the mammalian brain. Tinkering could have enabled this basic circuit to integrate more neural processes into the homeostatic regulation of sleep. Similarly, the research on the circadian mechanisms controlling sleep revealed a network. In mammals the master circadian clock is localized in the SCN. But the project of understanding the organization of the SCN and how different neural populations regulate circadian behavior in other parts of the organism is still in its early stages. The clock network in the fly may reveal design principles that are conserved even as the master clock was, in this case, brought together in one nucleus. Some support for this is provided by the hypotheses that there are morning and evening oscillators in both flies and mammals. The research on the clock network may provide a useful model for how different populations of morning and evening oscillators act to regulate different sub-mechanisms involved in sleep.

Adopting the fly as providing a model of the ur-mechanisms regulating sleep and using those as models for the mechanisms in us is a heuristic. Like all heuristics, it can fail. Even if it fails, it can serve an important role in initiating search for com-

ponents of the mechanism in the target organism. If it succeeds, it will reveal conservation at the level of mechanism design. The prospect of success explains the considerable research efforts, only some of which I have discussed, to understanding the homeostatic and circadian sleep mechanisms in the fly.

Conclusion

In this paper I have used recent research on sleep in flies to illuminate why researchers sometimes seek to investigate a phenomenon in a phylogenetically very distant organism—it can provide a simpler version of the mechanism. Evolution is a process that continually tinkers with mechanisms, often obscuring the basic principles that enable the mechanism to produce a given phenomenon. Looking to distantly related organisms that may have undergone less accretion of additional components enables researchers to peel back the history of evolutionary tinkering to identify the core mechanisms. Researchers can invoke the understanding they develop from simpler model organisms as heuristic guides to developing an understanding of the more complex mechanisms operative in target organisms such as humans.

There are a host of challenges researchers have to overcome in using relatively simple, phylogenetically distant organisms as models for mechanisms. Research on sleep in flies illustrates how researchers have overcome some of these challenges and are confronting others. In the case of sleep, researchers first had to change back from EEG recordings of brain rhythms as the means of identifying sleep to behavioral measures that could be applied to flies (and other animals). They could then investigate the mechanism in flies and appeal to conservation between the simpler model in flies and the target in humans to employ the results with flies as heuristic guides. Conservation is most commonly assessed for genes and indeed some of the early successes in using flies as models for sleep was involved determining the operations performed by proteins associated with conserved genes. Researchers could infer that the homologs in the target organism made the same contributions to sleep mechanisms. But, as I argued, the mechanisms generating sleep involved operations at a level above the molecular level. It required understanding the contributions of different populations of neurons.

I have provided examples of how research on flies is enabling researchers to develop accounts of mechanisms responsible for both homeostatic and circadian regulation of sleep. Noting that neural populations in the fly cannot be mapped directly onto neural structures in vertebrates, I raised a challenge for using research on these sleep mechanisms in flies as models for those in us. I have proposed, though, that although neural components cannot be mapped directly between flies and humans, the basic design of the mechanisms may be conserved. One will only be able to assess whether there is design conservation in this case if future researchers actually succeed in using models of the circuits in flies to identify components of the sleep mechanisms in us. But there is, as I have described, an energetic research program that is betting that the strategy will prove illuminating.

Declarations

Competing interests The author received no funding to support this research and reports no financial or non-financial interests that are directly or indirectly related to the work submitted for publication.

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