To sleep, perchance to live

Sleeping is vital for health, cognitive function, memory and long life

That "sleep is for the weak" has been the *Zeitgeist*—at least among students—for many years, but the effects of sleep deprivation are actually so damaging that it is now prohibited as a method of interrogation in most countries. A loss of resolve is just one of the serious emotional, cognitive and metabolic problems that the prolonged disruption of sleep can cause, which highlights its importance to our physical and emotional wellbeing. A recently published study of more than 10,000 UK civil servants, conducted over a 20-year period, found that participants who had reduced their sleep from the recommended seven hours per night to five or less were almost twice as likely to die from any given cause, and more than twice as likely to die from a cardiovascular problem (Ferrie *et al*, 2007).

Yet many of the underlying molecular and signalling processes of sleep remain poorly understood, despite significant progress in elucidating the physiological role of sleep and the sometimes lethal effects of complete sleep deprivation. In fact, sleep is so important—even to animals that can temporarily make do without it—that it has been conserved throughout evolution despite persistent selective pressures against it; sleeping makes an organism vulnerable to predators.

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Perhaps partly to minimize this vulnerability, the brains of some animals remain active during sleep, although the muscles relax and metabolic processes, including the heart rate, slow down. At first sight, this is surprising, especially in primates including humans whose brains consume a lot of energy. If the only requirement of sleep were to conserve energy, animals would be better off staying awake and alert while relaxing—a state that some humans achieve in meditation. The implication, then, is that sleep is about more than just muscle relaxation or energy conservation, and there is now accumulating evidence of its role both in maintaining the immune system—through mechanisms such as cytokine signalling—and in optimizing cognitive functions, especially memory, notably through communication between the cortex and hippocampus. In particular, sleep is directly involved in the consolidation of memory from short to long term in humans.

Understanding the event of sleep itself in a wide range of organisms, from the worm to the human, is no simple task, and many researchers have adopted a definition based on low-level brain activity that applies only to vertebrates. The French-born scientist Emmanuel Mignot, Director of the Centre for Narcolepsy at Stanford University (CA, USA), who studies sleep in the zebrafish, describes the event as: "extended periods of immobility with increased arousal threshold and most often a posture at the bottom or the top of the tank".

But this definition of vertebrate sleep does not necessarily describe the resting state of more primitive invertebrates. Not surprisingly, then, researchers studying organisms such as the roundworm *Caenorhabditis elegans* use different definitions. Roundworms enter a state of behavioural quiescence called lethargus before each of their four moults. A recent study carried out at the University of Pennsylvania School of Medicine (Philadelphia, PA, USA) found that the worms were less responsive during this sleep-like state, and were more likely to enter the state quickly and remain in it for longer after being deprived (Raizen *et al*, 2008). Perhaps more importantly, the study also found that the timing of lethargus coincided with synaptic plasticity and the formation of new neuronal connections, possibly promoting changes in the nervous system (Raizen *et al*, 2008).

Yet, worms and many invertebrates do not sleep in any sense that we might recognize and also fail to exhibit the patterns of electrical activity observed in the brains of sleeping mammals. As such, they are too primitive to have genes that might be associated with human sleep disorders. Scientists have therefore turned their attention to another familiar model organism, *Drosophila melanogaster*, to look for these genes. *Drosophila* enter a quiescent state for 8–14 hours per day in which they are immobile and slower to respond to stimuli—a period that diminishes with age, as in mammals and humans. Moreover, sleep in *Drosophila* bears many of the characteristics of mammalian sleep, including inter-individual variability, modulation by the same stimulants and hypnotics, and both changes in brain electrical activity and varying gene expression in sleeping and waking states (Ho & Sehgal, 2005; Cirelli & Bushey, 2008).

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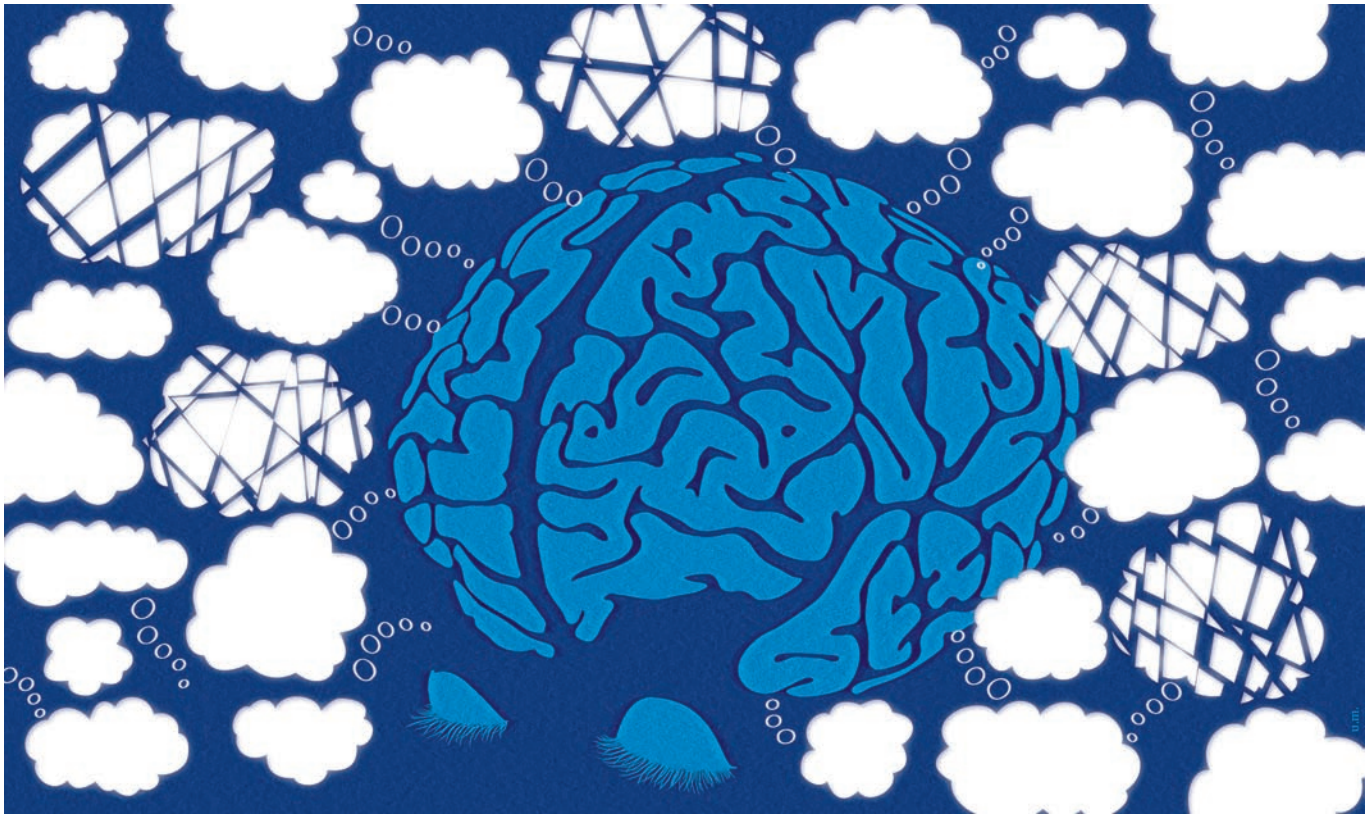
Given this similarity, *Drosophila* is a good model in which to look for genes that regulate sleep and wakefulness (Hendricks & Sehgal, 2004; Ho & Sehgal, 2005). A study in *Nature* conducted by researchers at the University of Wisconsin–Madison (WI, USA), for example, found that a fly mutant with an inactive version of the gene *shaker*—which causes the fly's legs to shake, along with other aberrant movements—stayed in this sleep-like state for only one-third of the usual time without apparent immediate ill effects, although with a shortened lifespan (Cirelli *et al*, 2005). The *shaker* gene itself encodes a voltage-dependent potassium channel that has a role in the conductance of electrical charges across neurons, but *minisleep* flies have an allele of *shaker* that leads to reduced conductance, causing large-scale electrical changes that can be measured by electroencephalography. The



study therefore raised two interesting questions: are disruptions in ion channels associated with sleep disorders such as narcolepsy and apnoea in mammals; and, is there a universal link between duration or quality of sleep and lifespan?

To answer these questions, the Wisconsin team turned to mice in which the role played by *shaker* in fruit flies is shared between 16 genes. The researchers focused their attention on the mouse gene *Kcna2*, which has the greatest sequence similarity to *shaker*, and found that mice with this gene knocked out were awake for around 20% longer and grew more slowly during their first two weeks of life (Douglas *et al*, 2007). Furthermore, although there were initially no obvious signs of altered electrical activity, the mice were prone to seizures and had an average lifespan of just 17 days, which indicates that the link between sleep and lifespan is also present in mammals.

However, some aspects of sleep regulation have changed markedly during the course of vertebrate evolution. As Mignot's work at Stanford has shown, there is a need for caution when extrapolating directly from one species to another. His studies with zebrafish have focused on the role of the molecule hypocretin, which is involved in promoting wakefulness and causes narcolepsy in humans when disrupted. Mignot created fish in which the gene coding for the hypocretin receptor was knocked out; this, he expected, would cause something similar to narcolepsy in the mutants. However, the effect was surprising: knocking out the receptor increased waking time (Yokogawa *et al*, 2007).

Although the exact explanation remains to be determined, Mignot has shown that the zebrafish sleep regulatory system involves radically different gene expression patterns to that of other vertebrates and responds more to light than to an underlying homeostatic clock-based control. It seems that, within the zebrafish system, hypocretin is involved in the promotion of sleep rather than wakefulness. But, although the specific action of hypocretin is different in zebrafish to that in humans, Mignot pointed out that his research does indicate that the hypocretin system has an important role in sleep regulation in most, if not all, vertebrates.

Animal models have also proven useful in elucidating how sleep can have an impact on health and the immune system. Recent research, carried out in fruit flies, has revealed a type of 'yin and yang' effect that results from a two-way relationship between circadian rhythms and innate immunity. *Drosophila* infected with the bacterium *Streptococcus pneumoniae* have disrupted circadian rhythms, which results in swings between hyperactivity and lethargy with occasional, sporadic sleep. Conversely, flies deprived of a gene called *timeless*, which regulates circadian rhythms, sleep soundly but are immunodeficient and die much more quickly when infected by the same bacterium (Shirasu-Hiza *et al,* 2007).

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Their results led the researchers to speculate that the infected flies are trapped in a fatal loop in which pathogenesis causes a loss of circadian rhythm, which, in turn,

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amplifies pathogenicity leading eventually to death. Moreover, the study is of relevance to humans claims one of its authors, Mimi Shirasu-Hiza from the Department of Microbiology and Immunology at Stanford University. "I absolutely think this work is applicable to humans […] In fact, parts of the immune system, like leukocyte activity, are already known to have circadian rhythm—that is, oscillate over 24 hours," she said. "There are many possible molecular signals to control these activities but it's often difficult to nail down exact causality in complex vertebrate systems. But, because the signalling pathways for both circadian biology and innate immunity are so highly conserved between flies and vertebrates, information from the fly is quite likely to help us understand how things work in humans," she said.

Yet, the need for caution in applying results from one species to another remains. This is most obvious in mammals, in which the role of sleep is greatly expanded compared with lower vertebrates and invertebrates. For humans in particular, regular, quality sleep has become so important that higher-level intellectual functions are severely impaired without it. This has led to speculation that sleep is intrinsically essential to the operation of a sophisticated brain and that this is why humans are more dependent on it than most mammals. However, Thomas Hahn, a leading brain researcher at the Max Planck Institute for Medical Research in Heidelberg, Germany, dismisses this idea. "Sleep duration and structure are evolved traits, and to a large degree reflect the evolutionary history of man," he said. "In that sense, humans are tuned to sleep [as much as they do], and physiological processes are tuned to match that pattern […] So I doubt very much that there is any fundamental cause for us sleeping this much."

This argument might be true, but is irrelevant to the study of how it works at a physiological level. Hahn also has clear opinions in this regard and argues that there is not much future in trying to unlock the secrets of the human brain and sleep through a purely bottom-up approach. "We think that in order to understand 'systems'—like the memory system—we should look at the interactions of the systems involved, and not only at the components, [which are] mostly single neurons and their physiology," he said. "The major reason for this is that nervous tissue is extremely heterogeneous; there are an extremely large number of components—billions of neurons—and many feedback loops. Therefore, predicting the system's behaviour from knowledge of its components is very complicated if not impossible."

Sleep in most warm-blooded vertebrates occurs in two distinct phases: rapid eye movement (REM) sleep and non-REM sleep. The two forms are distinguished in several ways, including changes in cortical electrical activity, which oscillates at a frequency of around 5 Hz during REM sleep and 0.5–2 Hz during non-REM sleep. For this reason, non-REM sleep is sometimes referred to as slow-wave sleep.

In humans, REM sleep accounts for around 20% of total sleep time and occurs four or five times a night. REM sleep is also characterized by the rapid eye movements for which it is named and an irregular heart beat; it is also the period in which the most vividly remembered dreams usually occur. As mammals only enter REM sleep after they have fallen asleep, it is possible to test the effects of REM sleep deprivation without affecting non-REM sleep. The overall impact of both can be detected by depriving animals first of all sleep and then specifically of REM sleep.

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Rats deprived only of REM sleep eventually develop the characteristics of total sleep deprivation—cognitive impairment, an increased heart rate, decreased body temperature, the development of ulcers and ultimately death—although not as quickly as rats deprived of all sleep. Taken together with the observation that some animals have evolved the ability to cope with only irregular snatches of REM asleep—such as migratory birds and some sea mammals—these findings suggest that although both types of sleep are important, it is non-REM sleep that is crucial for the day-to-day regulation of immunity, cognitive functions and probably development. This idea is strengthened by studies of the link between non-REM sleep and memory consolidation in humans, which have shown that although human performance at both mechanical and cognitive tasks is enhanced by sleep in general, non-REM sleep is most crucial (Marshall *et al*, 2006).

Nevertheless, it is difficult to untangle the role of both types of sleep in memory consolidation. The German philosopher Friedrich Wilhelm Nietzsche (1844–1900) famously argued that we cannot prove whether we genuinely forget things; all we can say is that we sometimes fail to remember what is in our brains. Philosophy aside, the traditional view of memory formation has been that memories recalled initially in the hippocampus are driven to the cortex and consolidated during non-REM sleep. However, recent work published in *Nature Neuroscience* suggests that it is the other way round and that the cortex is in charge, choosing which memories to select and which to discard (Mehta, 2007).

"Unlike most theories of consolidation, which suggested that memories are transferred from the hippocampus to the neocortex for long term storage during sleep, our data suggests that sleep may be erasing memories, thereby improving the signal to noise ratio between the relevant and irrelevant memories, and improving the memory capacity of the network," commented Mayank Mehta, an Assistant Professor in the Department of Neuroscience at Brown University (Providence, RI, USA) and author of the paper. "These new findings raise the possibility that during the slow wave sleep oscillations, the neocortex may be erasing memories from the hippocampus […] This can explain most of our findings in the past ten years, which show that the recently learned memory trace in the hippocampus seems to be getting erased after a period of sleep."

Yet, as ever in the realm of sleep and neuroscience, each answer begs a new question—in this case: how does the cortex decide which memories to erase? It is clearly not completely random, as people tend to remember things in which they are interested; therefore, emotion, motivation and inclination must have a role. The topic remains open for debate, not only in terms

of molecular mechanisms or complete systems, but also for higher-level states of the brain driven by emotion.

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However, what is clear is that the most definite progress in understanding sleep has been made at the systems level. Although some pieces of the puzzle are emerging at the molecular level, there is currently little consensus about how they all fit together. On this subject, as with the link between sleep and immunity, it is suitable to paraphrase the great English mathematician and physicist Sir Isaac Newton (1643–1727): we are still children playing on the seashore while the great ocean of sleep lies undiscovered before us.

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