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NEUROSCIENCE

Perchance to Prune

During sleep, the brain weakens the connections among nerve cells, apparently conserving energy and, paradoxically, aiding memory

By Giulio Tononi and Chiara Cirelli

EVERY NIGHT, WHILE WE LIE ASLEEP, BLIND, DUMB AND ALMOST PARALYZED, OUR brains are hard at work. Neurons in the sleeping brain fire nearly as often as they do in a waking state, and they consume almost as much energy. What is the point of this unceasing activity at a time when we are supposedly resting? Why does the conscious mind disconnect so completely from the external environment while the brain keeps nattering on?

The brain's activity during rest likely serves some essential function.

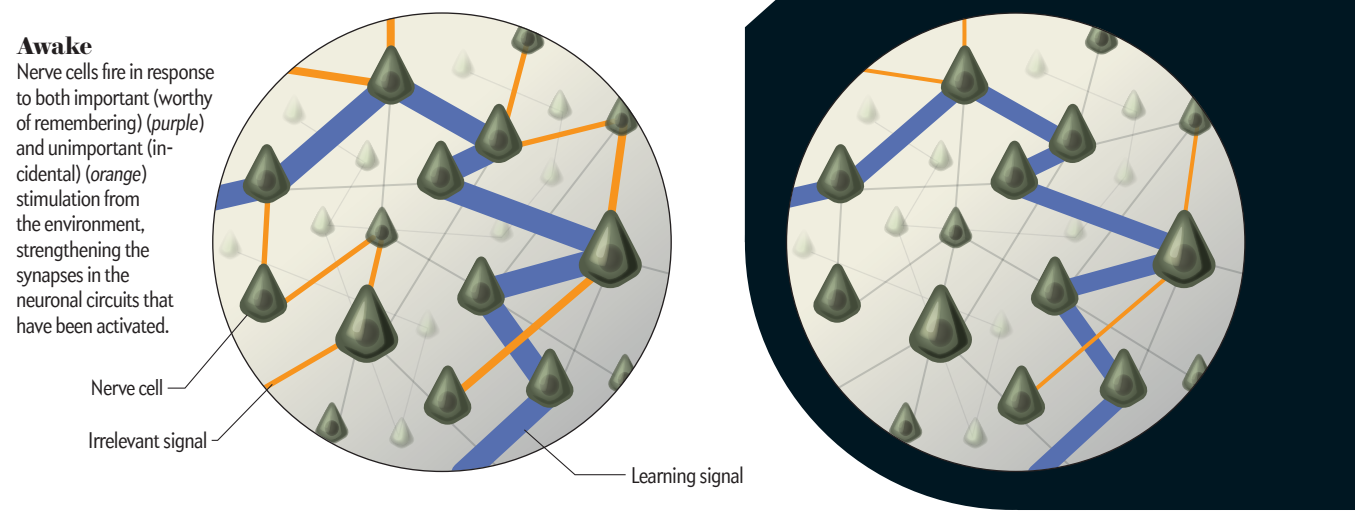
IN BRIEF

Sleep must serve some vital function because all animals do it.
Evidence suggests that sleep weakens the connections among nerve cells, which is a surprising effect, considering that strengthening of those connections during wakefulness supports learning and memory.
But by weakening synapses, sleep may keep brain cells from becoming oversaturated with daily experience and from consuming too much energy.

Photograph by Fredrik Broden

Why We Sleep

When we are awake, memories form as neurons that get activated together strengthen their links (*below left*). Sleep researchers have assumed that during sleep, reactivation of these neuronal circuits reinforces the links. But just the opposite may occur (*panel at right*): mounting evidence suggests that spontaneous firing during sleep may weaken the synapses, or contact points, between neurons in many roused circuits. Such weakening, the authors propose, would return the synapses to a baseline level of strength—a change that would conserve energy in, and reduce stress on, nerve cells. This return to baseline, called synaptic homeostasis, could be the fundamental purpose of sleep.



The evidence for this importance starts with sleep's ubiquity. All animals apparently sleep even though being unconscious and unresponsive greatly raises the risk of becoming another creature's lunch. Birds do it, bees do it, iguanas and cockroaches do it, even fruit flies do it, as we and others demonstrated more than a decade ago.

Furthermore, evolution has devised a few extraordinary adaptations to accommodate sleep: dolphins and some other marine mammals that must surface often to breathe, for example, sleep by alternately switching off one hemisphere of their brain while the other remains in a waking state.

Like many scientists and nonscientists, the two of us have long wondered what benefit sleep provides that makes it so crucial to living creatures. More than 20 years ago, when we worked together at the Sant'Anna School of Advanced Studies in Pisa, Italy, we began to suspect that the brain's activity during slumber may somehow restore to a baseline state the billions of neural connections that get modified every day by the events of waking life. Sleep, in this telling, would preserve the ability of the brain's circuitry to form new memories continually over the course of an individual's lifetime without becoming oversaturated or obliterating older memories.

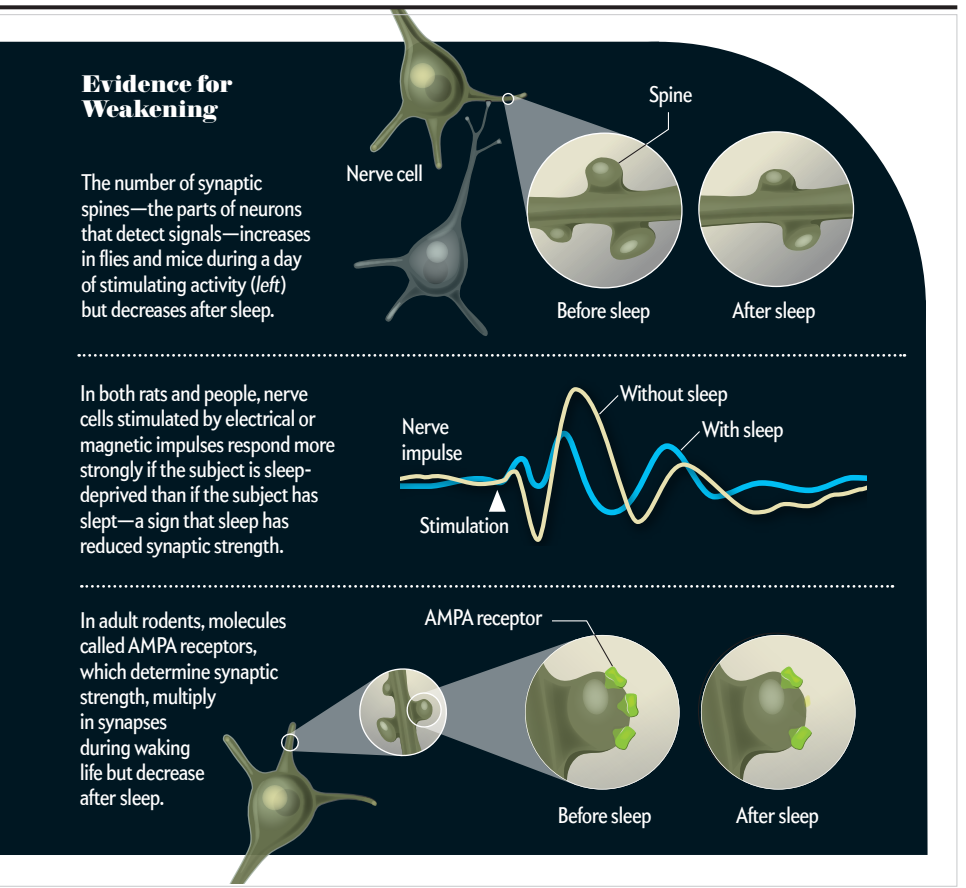
We also have an idea of why awareness of the external environment must be shut off during sleep. It seems to us that conscious experience of the here and now has to be interrupted for the brain to gain the chance to integrate new and old memories; sleep provides that respite.

Our hypothesis is somewhat controversial among our fellow neuroscientists who study sleep's role in learning and memory because we suggest that the return to baseline results from a weakening of the links among the neurons that fire during sleep. Conventional wisdom holds, instead, that brain activity during sleep *strengthens* the neural connections involved in storing newly formed memories. Yet years of research with organisms ranging from flies to people lend support to our notions.

SCHOOL OF NOD

SCIENTISTS first proposed the idea that sleep is important to memory nearly a century ago, and plenty of experiments since then have shown that after a night of sleep, and sometimes just a nap, newly formed memories "stick" better than they would if one had spent the same amount of time awake. This pattern holds for declarative memories, such as lists of words and associations between pictures and places, as well as for procedural memories, which underlie perceptual and motor skills, such as playing a musical instrument.

The evidence that sleep benefits memory led scientists to look for signs that the brain rehashes newly learned material at night. They found them: studies performed over the past 20 years, first in rodents and then in humans, show that patterns of neural activity during sleep sometimes do resemble those recorded while subjects are awake. For example, when a rat learns to navigate a maze, certain neurons in a part of the brain called the hippocampus fire in specific sequences. During subsequent sleep, rats "replay"



these sequences more often than predicted by chance.

Because of such findings, many researchers came to assume that sleep "replay" consolidates memories by further reinforcing synapses—the contact points between neurons—that have been strengthened when an individual is awake. The idea is that, as linked neurons fire repeatedly, the synapses connecting them more readily convey signals from one neuron to another, helping neuronal circuits to encode memories in the brain. This process of selective strengthening is known as synaptic potentiation, and it is the favored mechanism by which the brain is thought to accomplish learning and remembering.

Yet while replay and potentiation are known to occur during waking activities, scientists have so far found no direct evidence that the synapses in replayed circuits get strengthened during sleep. This lack of evidence hardly surprises us. It is consistent with our suspicion that while the sleeper lies unaware, all that brain activity—the "replay" as well as other, seemingly random firings—might actually be *weakening* neural connections, not strengthening them.

THE PRICE OF PLASTICITY

THERE ARE MANY good reasons to propose that synapses must become weakened as well as strengthened for the brain to function properly. For one thing, strong synapses consume more energy than weak ones, and the brain does not have infinite stores of energy. In humans the brain accounts for almost 20 percent of the body's energy budget—more than any other organ by weight—and at least two thirds of that portion goes to supporting synaptic activity. Building and bolstering synapses is also a major source of cellular stress, requiring cells to synthesize and deliver components ranging from mitochondria (the cell's power plants), to synaptic vesicles (which ferry signaling molecules), to various proteins and lipids that are needed for communication across synapses.

It seems clear to us that this strain on resources is unsustainable. The brain cannot go on strengthening and maintaining revved-up synapses both day and night for the whole of an individual's lifetime. We do not doubt that learning occurs mainly through synaptic potentiation. We simply doubt that strengthening continues to happen during sleep.

In contrast, synaptic weakening during sleep would restore brain circuitry to a baseline level of strength, thereby avoiding excessive energy consumption and cellular stress. We refer to this baseline-restoring function of sleep as preserving synaptic homeostasis, and we call our overall hypothesis about the role of sleep the synaptic homeostasis hypothesis, or SHY. In principle, SHY explains the essential, universal purpose of sleep for all organisms that do it: sleep restores the brain to a state where it can learn and adapt when we are awake. The risk we take by becoming disconnected from the environment for hours at a time is the price we pay for this neural recalibration. Most generally, sleep is the price we pay for the brain's plasticity—its ability to modify its wiring in response to experience.

But how does SHY explain sleep's salutary effects on learning and memory? How can weakened synapses improve the overall retention of skills and facts? Consider that, over the course of a typical day, almost everything you experience leaves a neural trace in the brain and that the significant events, like meeting a new person or learning a piece of music on the guitar, make up just a trifling portion of that neural encoding. To improve memory, the sleeping brain must somehow distinguish the "noise" of irrelevant information from the "signal" of significant happenings.

We suggest that in sleep, the spontaneous firing of neurons in the brain activates many different circuits in many different combinations, encompassing both new memory traces and old networks of learned associations. (You get a glimpse of this neural free-for-all in dreams.) The spontaneous activity lets the brain try out which new memories fit better with stored memories of proved significance and weakens those synapses that do not fit well in the grand scheme of memory. We and other investigators are exploring possible mechanisms by which brain activity could selectively weaken synapses that encode the "noise" while preserving those that correspond to the "signal."

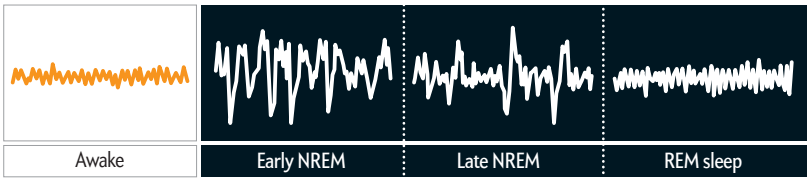
While the brain tries out these imaginary scenarios and enacts weakening

Illustrations by Jen Christiansen

POSSIBLE MECHANISM

Sleep Comes in Waves

Recordings of electrical activity in the brain show that brain waves undergo distinctive changes throughout the night as the sleeper alternates between rapid eye movement (REM) and non-REM (NREM) sleep (*graphs*). The slower waves of NREM sleep decrease in size through the course of a night—a pattern suggesting that the synapses involved in these waves get weaker. The authors propose that this weakening occurs in part because certain chemicals needed for strengthening activated synapses become much less concentrated then.



where appropriate, we had best be unaware of the surrounding environment and be incapable of acting in it; that is, we had best be asleep. Likewise, restoring synaptic homeostasis should not take place while we are awake because the events of the day would dominate the process, giving salience to them rather than to all the knowledge the brain has accumulated over a lifetime. The profound disconnection of sleep frees our brain from the tyranny of the present, creating an ideal circumstance for integrating and consolidating memories.

A WEAK CONNECTION

OUR PROPOSAL that the brain uses neuronal firing during sleep to weaken rather than strengthen synapses is supported in part by close analyses of data from a standard workhorse of sleep research: the electroencephalogram, or EEG. EEGs record patterns of electrical activity in the cerebral cortex via electrodes attached to the scalp. Decades ago EEG recordings of the sleeping brain revealed two main categories of sleep, called rapid eye movement (REM) and non-REM (NREM), that alternate throughout the night. Each has distinctive brain-wave patterns. In addition to the jittering of eyeballs underneath closed lids that gives REM sleep its name, that stage is dominated by relatively fast oscillations—quick ups and downs in the curves of the EEG readout, resembling EEG recordings of the waking state. In contrast, slow oscillations—with frequencies of about one cycle per second—are the most prominent feature of NREM sleep.

A decade ago the late Mircea Steriade of Laval University in Quebec discovered that the slow oscillations of NREM sleep arise when groups of neurons fire together for a little while (so-called on periods), then fall silent for about a fraction of a second (off periods) and then resume their synchronized firing. This was one of the fundamental discoveries in sleep research. Since then, scientists have also discovered that in birds and mammals, the slow waves are large if preceded by a long period of wakefulness and become smaller as sleep goes on.

We reasoned that if synapses are strong, neurons will synchronize their firing more, producing larger slow waves. If synapses are weak, neurons will be less synchronized and the resulting slow waves will be smaller. Results of computer simulations and experiments in humans and animals led us to conclude that the big, steep slow waves early in the night indicate that synapses have been strengthened by prior wakefulness, whereas the small, shallow slow waves early in the morning indicate that synapses have become weaker during sleep.

Direct support for the idea that synapses become weaker during sleep, and may even be pruned away, comes from studies in animals. In fruit flies, for instance, we find that sleep reverses a progressive increase in the number and size of synapses that occurs during the day, especially when the flies are exposed to stimulating environments. Synaptic spines are specialized protrusions on a neuron's signal-detecting arm. When fruit flies spend the day interacting with other flies, neurons throughout their brain sprout more synaptic spines by evening than were present in the morning.

Just as remarkably, the number of spines goes back to the baseline level by the following morning if—and only if—the flies are allowed to sleep. We saw a similar phenomenon in the cerebral cortex of adolescent mice: the number of synaptic spines tended to rise when the animals were awake and to fall when they slept. In adult rodents, the upshot is the same, although it is not the number of synaptic spines that changes with wakefulness and sleep but rather the abundance of certain spine molecules, known as AMPA receptors, that determine the strength of a synapse. When we monitored these AMPA receptors, we found that their number per synapse increases after wakefulness and decreases after sleep. More receptors make for stronger synapses; fewer mean the synapses have weakened.

Synaptic strength can be gauged directly by using an electrical probe to stimulate neural fibers in the cortex. The neurons respond with an induced electrical discharge that is larger when synapses are strong and smaller when the connections are weak. We showed that in rats, stimulated neurons fire more strongly after a few hours of wakefulness and less strongly after sleep. Marcello Massimini of the University of Milan in Italy and Reto Huber, now at the University of Zurich, performed a similar experiment in humans. Instead of an electrical probe, they turned to transcranial magnetic stimulation—a short magnetic pulse applied to the scalp—to stimulate the underlying neurons. They then recorded the strength of the cortical responses with high-density EEG. The results were clear: the longer a subject was awake, the larger the EEG responses. It took a night of sleep for cortical responses to return to the baseline.

LESS IS MORE

THE COMMON conclusion of these experiments, which we performed over two decades, is that spontaneous cortical activity in sleep does indeed weaken the synaptic connections in neural circuits, whether by damping their ability to send electrical impulses or by erasing them outright.

This process, which we call down selection, would ensure the survival of the circuits that are "fittest," either because they

were activated strongly and consistently during wakefulness (say, by playing the right notes on a guitar while trying to master a new piece) or because they were better integrated with previous, older memories (as would be the case for a new word encountered in a known language). Meanwhile synapses in circuits that were only mildly enhanced during wakefulness (such as fumbled notes on the guitar) or that fit less with old memories (such as a new word presented in an unknown language) would be depressed.

Down selection would ensure that insignificant events would leave no lasting trace in our neural circuitry, whereas memories of note would be preserved. As an additional bonus, down selection would also make room for another cycle of synaptic strengthening during wakefulness. Indeed, some findings imply that among its many other benefits for learning and memory, sleep aids the subsequent acquisition of new memories (material encountered before the next bout of sleep). Quite a few studies have shown that after a night of sleep, you can learn new material much better than you can after having been awake all day. (Students, take note.)

Although we have no direct evidence for a mechanism that would produce selective weakening of activated synapses as yet, we have a notion of how synaptic weakening could occur. We suspect the slow waves of mammalian NREM sleep somehow play a role. In lab studies of rat brain tissue, nerve cells became less effective at passing signals to one another when stimulated in ways that mimic the synchronized on/off cycles of slow-wave sleep.

The chemistry of the brain also changes in NREM sleep in a way that could lead to synaptic weakening. In the awake individual, a concentrated soup of signaling chemicals, or neuromodulators—including acetylcholine, norepinephrine, dopamine, serotonin, histamine and hypocretin—bathe the brain and bias synapses toward strengthening when signals pass through them. During sleep—especially NREM sleep—the soup becomes much less concentrated. This diluted milieu of neuromodulators may bias the neural circuitry so that synapses become weakened, rather than strengthened, when signals flow across them. The process might also involve a substance called brain-derived neurotrophic factor (BDNF), which is known to promote synaptic strengthening and to be involved in memory acquisition. BDNF levels are high in neurons during wakefulness and minimal during sleep.

LOCAL SLEEP

REGARDLESS of specific mechanisms and selective processes, the evidence is strong in several species that overall synaptic strength goes up during wakefulness and down during sleep: the core prediction of SHY. We can test SHY further by examining some of its intriguing corollaries.

For example, if the hypothesis is correct, then the more plasticity a part of the brain undergoes during wakefulness, the more that part should need to sleep. "Sleep need" can, in turn, be indicated by an increase in the size and duration of NREM slow waves. To explore this prediction, we asked human subjects to learn a novel task: how to reach a target on a computer screen while the cursor (controlled by a mouse) is systematically rotated. The part of the brain that engages in this kind of learning is the right parietal cortex. Sure enough, when our subjects slept, the slow waves over their right parietal cortex were larger, relative to waves from the same area on the night before learning occurred. These large waves did flatten out in the course of the night, as such oscillations do. But those large, localized waves at the start of the night tell us that particular part of the brain had been exhausted by the task we assigned.

Many other experiments by the two of us and others have since confirmed that learning, and more generally the activation of synapses in circuits, produces a local increase in sleep need. Recently we have even found that prolonged or intense use of certain circuits can make local groups of neurons "fall asleep" even though the rest of the brain (and the organism itself) remains awake. Thus, if a rat stays awake longer than usual, some cortical neurons show brief periods of silence that are basically indistinguishable from the off periods observed during slow-wave sleep. Meanwhile the rat is running around, its eyes open, tending to its business, as any awake rat would do.

This phenomenon is called local sleep, and it is attracting scrutiny from other investigators. Our latest studies indicate that localized off periods also occur in the brains of sleep-deprived humans and that those periods become more frequent after intense learning. It seems that when we have been awake for too long or have overexerted certain circuits, small chunks of the brain may take quick naps without giving notice. One wonders how many errors of judgment, silly mistakes, irritable responses and foul moods result from local sleep in the brains of exhausted people who believe they are fully awake and in complete control.

SHY also predicts that sleep is especially important in childhood and adolescence, times of concentrated learning and of intense synaptic remodeling, as many studies have shown. In youth, synapses are formed, strengthened and pruned at an explosive rate never approached in adulthood. It makes sense that down selection during sleep would be crucial to minimize the energy costs of this frenzied synaptic remodeling and to favor the survival of adaptive neural circuits in these stages of life. One can only wonder what happens when sleep is disrupted or insufficient during critical periods in development. Might the deficit corrupt the proper refinement of neural circuits? In that case, the effect of sleep loss would not merely be occasional forgetfulness or misjudgment but a lasting change in the way the brain is wired.

We look forward to testing SHY's predictions and exploring its implications further. For example, we hope to discover whether sleep deprivation during neural development leads to changes in the organization of brain circuitry. We would also like to learn more about the effect of sleep on deep-brain areas, such as the thalamus, cerebellum, hypothalamus and brain stem, and about the role of REM sleep in synaptic homeostasis. Perhaps we would then learn if sleep is indeed the price of waking plasticity, a price that every brain and every neuron must pay. SA

MORE TO EXPLORE

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SCIENTIFIC AMERICAN ONLINE

Watch author Giulio Tononi speak about the function of sleep at **ScientificAmerican.com/aug2013/sleep**