

The Sleeping Brain

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Editor's Note: The role of sleep has long baffled scientists, but the latest research is providing new indicators about what it does for both the brain and body. While scientists believe that sleep re-energizes the body's cells, clears waste from the brain, and supports learning and memory, much still needs to be learned about the part it plays in regulating mood, appetite and libido.

Why do we spend a third of our life asleep? The answer seems obvious: To recover from the “fatigue” of being awake, to be ready for another day of challenges, good or bad. All of us have experienced the consequences of a sleepless night: everything requires more effort; we lack energy and motivation, and feel groggy, irritable, and snappish.

But there is strong, objective proof that, far from being just such a “time filler,” sleep serves an active, essential function.¹ We know that all animal species that have been carefully studied sleep, with no exception.¹ If sleep were not essential, one would expect that some would have evolved to do without it, since time spent asleep reduces time spent foraging, reproducing, or monitoring the environment. Moreover, being asleep puts an animal in a potentially dangerous situation, because it reduces the ability to promptly respond to stimuli that signal threat. Thus, sleep makes little sense, from an evolutionary point of view, unless it provides enough essential benefits to overcome its inherent risks.

We also have learned that sleep is exquisitely regulated. There are complex mechanisms in our brain that increase the duration and/or the depth of sleep after sleep deprivation² to permit the recovery of some of what was lost (homeostatic regulation). There are also neural mechanisms that tend to consolidate sleep at a certain phase of the 24-hour cycle (circadian regulation): night for us and day for many rodents. Sleep loss or chronic sleep disruption also has many negative consequences, including adverse effects on metabolism and immune function.^{3,4}

The most obvious of these adverse effects are on the brain. Cognitive deficits of many kinds⁵ are apparent in humans, although with substantial inter-individual differences^{6,7} after just one night of total sleep deprivation or when sleep is cut short by several hours every night for a week or more.^{8,9} Attention, working memory, and the ability to learn and remember decline. When we are sleep deprived, it is more difficult to speak fluently, assess risks, and appreciate humor.^{8,9} Importantly, experiments have shown that these cognitive impairments can be reversed by sleep but not by the same period of quiet wakefulness.¹⁰ Similarly, there is evidence that cognitive deficits caused by

sleep loss at night can be prevented or delayed by naps.¹¹ The open question is: Why does sleep offer a special time for the brain's recovery?

Sleep versus Rest

In our search for an answer, we started by identifying the fundamental feature of sleep—what distinguishes it even from quiet, restful wakefulness: sensory disconnection. During quiet wakefulness, when we sit on a sofa in a silent and dark room after having exercised, for example, our muscles can recover from fatigue. Yet, we are still able to react and move promptly if the phone rings. In other words, we are still connected to the world. On the other hand, when deeply asleep our capacity to react to a mild stimulus—a noise coming from the next room, or that phone call—is reduced substantially.

Thus, any hypothesis about the essential function of sleep must take into account that, when asleep, we are essentially offline: sensory disconnection must be an essential requirement for whatever function sleep serves. If not, natural selection would likely have found a way to perform the same function while awake, avoiding the danger of being unable to monitor the environment.

Over the past 20 years, we have developed and tested a comprehensive hypothesis about the core function of sleep: The Synaptic Homeostasis Hypothesis (SHY).¹²⁻¹⁴ Briefly, SHY states that sleep is the price we pay for brain plasticity. According to our hypothesis, during wakefulness, synapses—the links that allow neurons to communicate with each other—undergo net strengthening (potentiation) as a result of learning. Learning is an ongoing process that happens all the time while we are awake, constantly adapting to an ever-changing environment. The remarkable and pervasive plasticity of the brain is an established fact and is essential for survival. However, it is a costly process, because stronger synapses increase the demand for energy and cellular supplies, lead to decreases in signal-to-noise ratios (because neurons would start responding less selectively to stimuli), and saturate the ability to learn.

This is where sleep enters the picture. According to SHY, while our brain is offline—disconnected from the environment—neural circuits can be reactivated, renormalizing synaptic strength. As

explained below, this renormalization favors memory consolidation and the integration of new with old memories, and eliminates the synapses that contribute more to the “noise” than to the “signal.” Just as importantly, synaptic renormalization during sleep restores the homeostasis of energy and cellular supplies, including many proteins and lipids that are part of the synapses, with beneficial effects at both the systems and cellular level.

Thanks for the Memories

It is useful to expand a bit on the rationale for SHY. First, the hypothesis emphasizes the obvious fact that wakefulness is the time when we can learn about our current environment, since only then do correlations in a neuron’s input reflect the causal structure of that environment. By the same token, sleep is not a good time for forming new memories, since we would run the risk of remembering fantasies and dreams, rather than events in the real world.

Second, learning is massive and ubiquitous throughout wakefulness: every waking minute you lay down innumerable neural traces about facts and events. Try the simple experiment of recalling everything that you saw, heard, and did today. You will realize that it is possible to remember innumerable facts and events about your daily activities, even if you did not start the day with the specific goal of memorizing them.

Third, learning during waking should occur primarily by synaptic potentiation, not depression; i.e. by strengthening rather than weakening the connections among neurons. Why? Because firing is much more energetically expensive than silence^{15,16} by default neurons that tend to fire very little.^{17,18} And since any decrease in firing starting from such low levels would be hard to detect, neurons should signal an important event by increasing their firing.

So how can a neuron, located deep in the brain without direct access to the external world, judge what events are potentially important? Simply by paying attention to those inputs (or synapses), out of the few thousands it receives, that are firing strongly and together. The increased firing of several inputs, more or less synchronously, represents a “suspicious coincidence” and is a good indicator that many of its input neurons had something important to signal, hence worth relaying further. During a waking

day, then, one can expect that many synapses will be strengthened throughout the brain, establishing memory traces.

But now a different issue arises. If the brain undergoes massive synaptic potentiation to establish memory traces during a waking day, there must be a way to ensure that synaptic strength does not grow indefinitely. Otherwise, neurons would quickly reach synaptic saturation, obliterating all memory traces, not to mention that energy and supplies would not be sustainable under these conditions. Clearly, there must be synaptic renormalization to regulate synaptic strength; a kind of synaptic homeostasis to prevent the nervous system from descending into chaos.

In this respect, the default assumption among neuroscientists has been that synaptic homeostasis is maintained during learning itself, when the brain is online. SHY, instead, proposes that synaptic renormalization should not happen during waking, when we are at the mercy of a particular environment and slaves of the “here and now,” but during sleep, when the brain is offline. Freed from the tyranny of its immediate environment, the brain can sample all its memories—old and new—and renormalize the total amount of synaptic strength in a smart way, preserving and consolidating those newly formed memory traces that fit best with its overall knowledge basis, while forgetting those that fit less well. Thus, sleep should be a time for net synaptic depression, leading to optimal “down-selection” of memory traces.

Testing a Hypotheses

How can the general idea underlying SHY be tested? There is no one comprehensive measure of synaptic strength, especially *in vivo* in freely moving animals. Over the years, we have employed many different methodologies, from continuous electrophysiological recordings in rodents and humans to molecular and genetic experiments in flies, mice, and rats. For instance, by using electrical stimulation (in rats) or transcranial magnetic stimulation (in humans, using a magnet above the skull to stimulate the brain non-invasively), we have found that the neural response triggered by a stimulus of fixed amplitude is bigger after several hours spent awake compared to several hours spent asleep,^{19,20} consistent with a net increase in synaptic strength during wakefulness.

These results are in line with SHY's predictions, but one cannot rule out that changes in neuronal excitability may also play a role in?21 More direct evidence comes from experiments in cortical slices, where we found that frequency and amplitude of miniature excitatory synaptic potentials increase after waking and decrease after sleep.²² Measuring the total amount of synaptic proteins in large regions of the fly and rat brain, we similarly found an increase after waking as compared to after sleep.^{19,23,24} Furthermore, in adolescent mice in which neural circuits are still being formed and refined, we found that during wakefulness the formation of new synapses exceeds their elimination, while the opposite is true during sleep.²⁵

Perhaps the most direct way to measure synaptic strength is through an ultrastructural approach that literally measures the size of the synapses. It is well established that stronger synapses are bigger, which implies that, per SHY, most synapses should grow with wakefulness and shrink with sleep, in a matter of a few hours. To test this prediction, we used serial block face electron microscopy (SBEM), a new method that permits the effective and automatic acquisition of high-resolution, tridimensional images of many synapses. Because the actual measurements of the synapses must be performed manually, it took several people four years to measure ~7,000 synapses in the mouse cerebral cortex.

At the end of this laborious process, the results were clear: six to eight hours of sleep led, on average, to an 18 percent decrease in the size of the synapses as compared to six to eight hours of non-forced wakefulness at night. Similar results were obtained when sleep was compared to six to eight hours of wakefulness enforced by exposure to novel objects during the day,²⁶ proving that these changes are due to behavioral state (sleep vs. waking) and not to circadian factors (day vs. night). The sleep-related decline in synapse size occurred in both areas that were examined—primary motor and primary sensory cortex.

Across the population of neurons, the decrease in synaptic size with sleep largely followed a scaling relationship, meaning that the change was proportional to the size of the synapse. Intriguingly, however, sleep-related downscaling showed some selectivity, occurring in the great majority of synapses (~80 percent) but sparing the largest ones, which may be associated with the most stable memory traces. The magnitude of these changes is worth considering. There are approximately 100 billion neurons in our

brain, 16 billion of them in the cerebral cortex alone (14 million in the mouse cortex), and each neuron receives thousands of synapses. Thus, if what we observed in two cortical areas extends to other brain regions, every night trillions of synapses in our brain could get slimmer by nearly 20 percent.

The SBEM study was recently published along with research from an independent group, which used biochemical and molecular methods to confirm SHY's prediction that synapses undergo a process of scaling down during sleep.²⁷ This study also showed that one gene, Homer1a, is important for the sleep-mediated downscaling process.

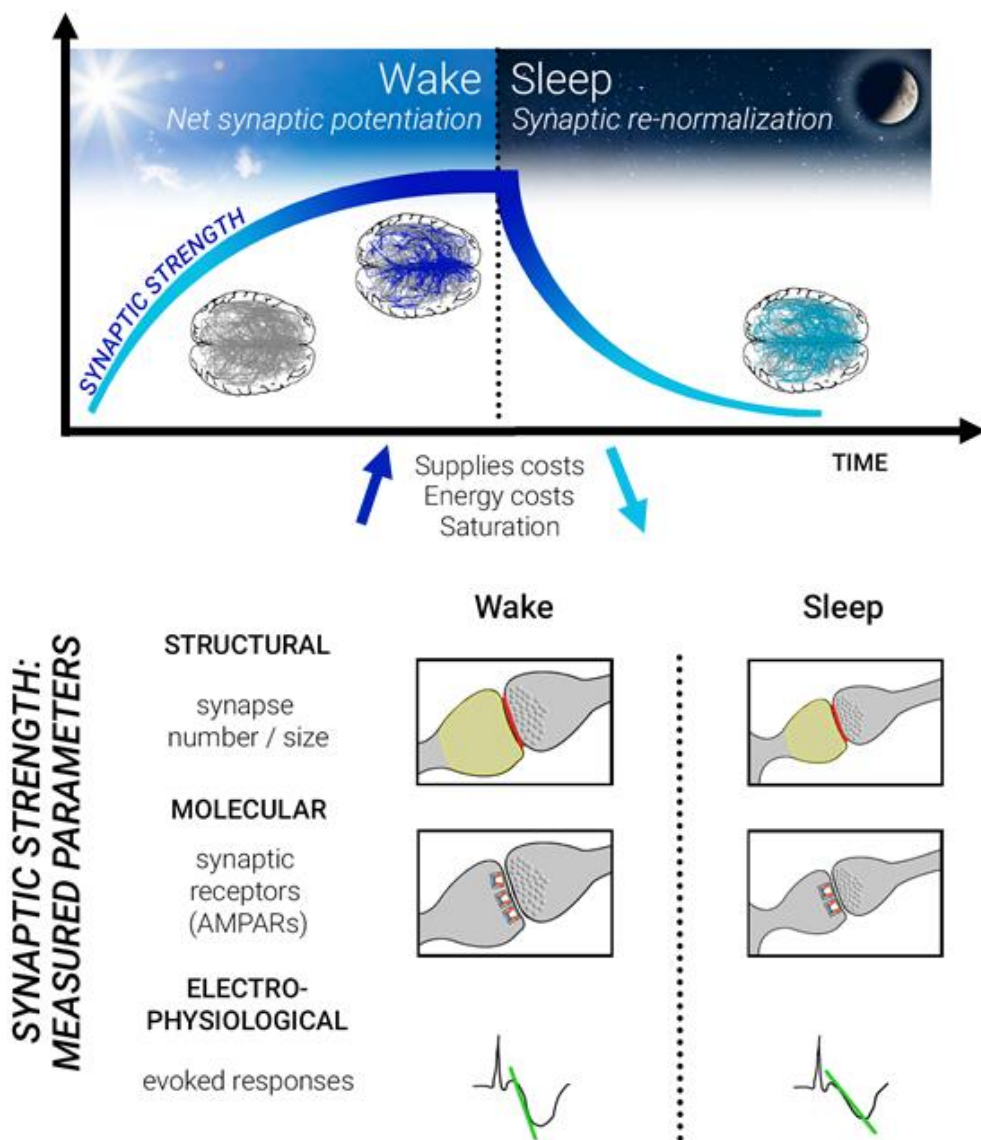


Figure 1. Top, Schematic diagram describing the main claim of SHY: net synaptic increase occurs during wake (during the day in humans and diurnal animals), when many circuits in the brain get potentiated (dark blue lines in the brain schematic), resulting in cellular and systems' costs, followed by synaptic renormalization during sleep, when most, if not all circuits undergo synaptic down-selection (light blue lines).

Bottom, Parameters used to test SHY. While structural and molecular measures more directly reflect synaptic strength, electrophysiological measures such as responses evoked by direct stimulation of the cerebral cortex can be strongly affected by other factors that modulate intrinsic neuronal excitability, including the levels of neuromodulators and the balance between excitation and inhibition, and thus cannot be used alone to infer synaptic strength. In the upper panel (structural), the red line indicates the axon to spine interface (ASI), and the yellow area outlines the head of the spine. In the middle panel (molecular), excitatory glutamatergic receptors (AMPA) are shown as squared boxes.

Avenues to Explore

We still do not know how synaptic renormalization occurs at the individual synapse level, which would require measuring changes across the sleep/wake cycle and before and after learning. SHY proposes that total synaptic strength is downscaled during sleep, but this does not mean that all synapses need to be renormalized every night. In computer simulations, we showed that various synaptic rules enforcing activity-dependent depression during sleep are compatible with the renormalization process predicted by SHY.

For example, it could be that stronger synapses are either depressed less than weaker ones²⁸ or are completely protected from depression.^{29,30} In the latter implementation of down-selection, when a neuron detects increased firing in many of its inputs during sleep (and thus fires strongly), the associated synapses will be protected from depression and maintain their current strength. The result is that, while the strength of these synapses does not increase in absolute terms, as is the case during wake, it does increase in relative terms, because the remaining synapses, those that were not protected, end up losing strength. This competitive down-selection mechanism has the advantage that synapses activated strongly and consistently during sleep survive mostly unchanged and may actually consolidate, becoming more resistant to interference and decay.

We believe that through this single process—synaptic renormalization—sleep can provide many of the benefits that behavioral studies have documented at the systems level: The ability to learn new things the next day, the consolidation of what has already been learned, and “smart” forgetting (i.e., the systematic elimination of irrelevant memory traces). However, much remains to be done. We plan to test whether the ultrastructural changes found in the cerebral cortex also happen elsewhere in the brain, such as in the hippocampus, another region crucial for brain plasticity and learning.

It is also essential to establish what happens during early development, when brain circuits are still being formed and synapses show a very high turnover. Additionally, we are trying to determine what might happen during sustained sleep loss: Do synapses keep growing as in normal waking, or do they start shrinking and disappearing, perhaps through a massive “pruning” process that is not as carefully regulated as during normal sleep? And if so, is abnormal pruning more pronounced during sensitive periods of development when neural circuits are still forming, perhaps with long-term consequences for the wiring of the adult brain?^{31,32}

Financial Disclosure

Bios

Chiara Cirelli, M.D., Ph.D., is a professor in the Department of Psychiatry at the University of Wisconsin–Madison. She received her medical degree and Ph.D. in neuroscience from the University of Pisa, Italy, where she began her investigation of the molecular correlates of sleep and wakefulness and the role of the noradrenergic system in sleep regulation. She continued this work at the Neuroscience Institute in San Diego as a fellow in experimental neuroscience, and subsequently at the University of Wisconsin. Cirelli’s research is aimed at investigating the fundamental mechanisms of sleep regulation by using a combination of molecular and genetic approaches.

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