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Disappearing Before Dawn

Gene expression studies are lending support to a new hypothesis for why everyone sleeps: to prune the strength or number of synapses.

By Kelly Rae Chi

At 10 a.m. on a frigid January, the lights automatically flicker on in a rat room at the University of Wisconsin-Madison's Research Park. Postdoc Erin Hanlon strolls in, still wearing her scarf from the trip to the lab, where she will spend the next hour or so with Telito, a rat. Telito's cage is tucked away in a television cabinet-like enclosure. He's freely moving but connected to a nearby computer by a bundle of wires emanating from the four tiny electrodes implanted into his cortex, held in place with screws and dental cement. She'll teach him to extend one paw through a plastic slot to grab a food reward—a task that will exercise a specific region of his brain. After 92 trials, she'll close the door behind her, let him nod off, and wait as the computer records the electrical brain waves of his slumber.

Hanlon is trying to replicate a similar 2004 experiment in humans performed by the same group, led by Chiara Cirelli and Giulio Tononi, which produced data that researchers are interpreting in two very different ways.[1]

In the experiment, the group asked human subjects to complete a motor task using a computer mouse while wearing a snug-fitting, high-density electroencephalogram (EEG) cap. After the participants performed the task, the researchers measured their sleep patterns. They noticed an interesting pattern in subjects' slow waves, electrical patterns of less than four waves per second that are thought to reflect the need for sleep. In general, people who are sleep-deprived tend to have more slow waves, and those waves are larger in amplitude than the slow waves of people who aren't sleep-deprived. In this experiment, slow waves were larger and occurred more often in the specific brain region used in the task, compared to other areas even within the same immediate brain region. And those subjects with the most active slow waves in that region seemed

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to perform better on the task the next day.

It was one of many experiments designed to answer one of life's biggest unsolved puzzles: Why do we sleep? For some researchers who study memory, the findings support a popular theory that the purpose of sleep is to replay and consolidate memories from the previous day. To them, sleep is important for memory, and the deep, slow waves seen in the same part of the brain used in a task indicate that the brain circuits involved in the task are reactivating. Such reactivation, or "replay," could explain why participants perform the task with greater accuracy after a night of sleep.

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But for Cirelli and Tononi, their findings suggested an entirely different—and controversial—theory was perhaps true.

Gene expression studies are lending support to a new hypothesis for why everyone sleeps: to prune the strength or number of synapses.

Sleep's core function, Cirelli and Tononi say, is to prune the strength or number of synapses formed during waking hours, keeping just the strongest neuronal connections intact. Synapse strength increases throughout the day, with stronger synapses creating better contact between neurons. Stronger synapses also take up more space and consume more energy, and if left unchecked, this process—which Cirelli and Tononi believe occurs in many brain regions—would become unsustainable.[2,3] Downscaling at night would reduce the energy and space

requirement of the brain, eliminate the weakest synapses, and help keep the strongest neuronal connections intact. This assumption is based on the principle in neuroscience that if one neuron doesn't fire to another very often, the connection between the two neurons weakens. By eliminating some of the unimportant connections, the body, in theory, eliminates background connections and effectively sharpens the important connections.

It's unclear how slow waves could affect synaptic strength at a molecular level, but Cirelli and Tononi suspect the slow-wave activity triggers a weakening of synapses, and the more slow waves, the more subsequent downscaling. Their belief stems from the timing of the slow waves, which swell early in the night and taper off. Plus, molecular and electrophysiological evidence indicates synapses are stronger at the beginning of the night and weakest after a long bout of sleep. To Cirelli and Tononi, the weakening of synapses overnight—which could also theoretically help people perform better on a task the next day—is the ultimate purpose of sleep.

Giulio Tononi and Chiara Cirelli
© Eric Tadsen

"At this point, it's a hypothesis that demands our attention," says Robert Stickgold, an associate professor of psychiatry at Harvard Medical School in Boston, who says he still supports the theory that the purpose of sleep is to replay and consolidate memories. "Insofar as it's true—and there's no really strong evidence that it isn't—it's going to shape our whole understanding of sleep."

Starting in the late 1980s, Cirelli and Tononi, who also live together, began experimenting with cats and rats to try to decipher the molecular mechanisms of sleep homeostasis in the brain. Others in the field were looking at the effect of sleep on the activity of neurons in discrete brain areas, such as the hypothalamus and brainstem. But if sleep was really a core need of the brain, Cirelli and Tononi reasoned that it should be reflected in the molecules across entire regions of the brain, like the cortex.

In 1989, Constantine Pavlides and Jonathan Winson from the Rockefeller University in New York tested the idea that the same daytime patterns of neuronal firing in the hippocampus—a brain region important for learning and memory—occur during sleep. To do this, they used electrodes to record activity in the hippocampus of rats while they explored a rectangular box. The researchers saw increased neuronal activity during the task, and saw the same increases during both slow-wave sleep (non-REM) and REM sleep,[4] suggesting the animal was replaying the memory of the task. It was around this time that the hypothesis emerged that sleep serves to replay and consolidate memories.

As more ideas came forward, many researchers began to think that sleep serves a variety of functions, such as conserving energy in the body, healing wounds, and synthesizing molecules that are depleted during the day. But Tononi found it hard to steer clear of the central issue, saying he was "romantically inclined" to believe that there might be a single core function of sleep. Over the next decade or so, he and Cirelli studied gene expression patterns, and their hypothesis began to emerge.

In the mid-1990s, they showed that genes coding for transcription factors such as c-fos, essentially markers of neuronal activation, are elevated in the later part of the waking day and lower during sleep in most brain regions. To them this finding indicated that broad changes in gene expression might occur across the brain during sleep. "That was the clue," says Cirelli. "If [transcription factors] change so dramatically between sleep and waking, that means that there are many other genes that can change between sleep and waking."[5]

"It's a hypothesis that demands our attention." — Robert Stickgold

At the time, Tononi was beginning to think about fundamental differences between sleep and wake states. Learning was an obvious choice, considering that animals learn while awake, not while asleep. Most forms of learning occur by strengthening of connections between neurons; this makes learning energetically costly to the brain. Stronger synapses consume more energy and space, and they require more cellular supplies, such as membranes to increase the surface area of contact (and chemical signaling).

According to Cirelli, neurons expend up to 80 percent of their energy on sending and receiving electrical signals, a process that adds and strengthens synaptic connections between neurons. This process could not continue indefinitely, they reasoned—at some point, the strength of those synapses would have to decrease. A global downscaling would shave off the weakest synapses, either in number or in size. This "pruning" would help sharpen the stronger connections, which, presumably, were more important in learning and retaining what you've learned.

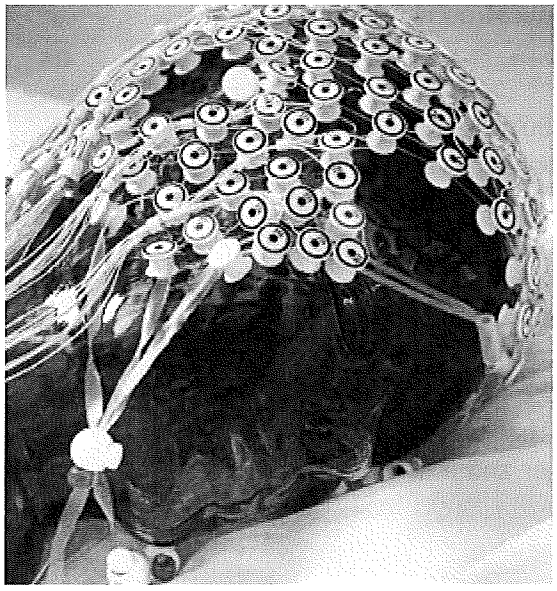
In 2000, they screened for the activity of 10,000 gene transcripts in the rat brain

to see which were associated with sleep. When they compared results from rats that were awake, sleep-deprived, or asleep for 8 hours, the scientists found 44 genes with increased expression during periods of wakefulness and/or sleep deprivation. Many of these genes were associated with synaptic plasticity, such as neurotransmitters and growth factors like brain-derived neurotrophic factor (BDNF).

Initially, Cirelli says, they were surprised at the findings. But then, as their hypothesis solidified, everything began to make sense. "Looking at this data in 2000, 2001, and the last one was in 2004, the pattern started coming out" that genes related to synaptic potentiation showed increased expression during waking hours, but not sleep, she says. Learning could not always be associated with stronger synapses, they reasoned—for one, sleep improves some aspects of learning, and synapses tend to weaken during sleep. Alternatively, the findings suggested, indirectly, that sleep was necessary to prune the number or strength of synapses down to baseline levels, and it is this process that boosts learning and memory. "It was not only true in the rat, but in the hamster and in the sparrows and mice that other people have described," she adds.

Today, Cirelli and Tononi's joint lab employs 20 people pulsing in and out of a central office room, which looks like the newsroom of a community newspaper with its stacks of papers and general chaos. Coming and going

at various times in the day and night, they are all wrestling with the synaptic homeostasis hypothesis in some way. As a result, their sleep patterns are as diverse as those of the systems they now study: humans, flies, and rats.



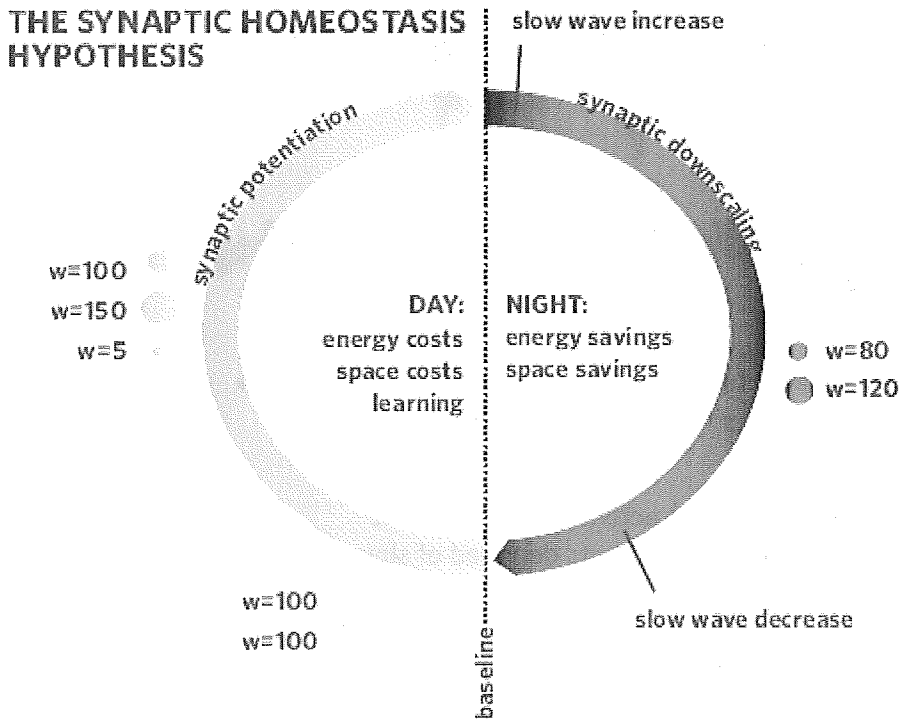
A high-density electroencephalogram (EEG). Copyright Eric Tadsen

In one of the lab's many rooms, postdoc Ugo Faraguna checks on Toufa the rat. Like Hanlon's rats, Toufa has electrodes recording his brain activity, but he also has a needle-sized tube implanted in the cortex so that Faraguna can inject him with potassium chloride. Faraguna was in the same room two years ago when he first stumbled on potassium chloride as a way to chemically enhance the strengthening of synapses. This finding, like many others, happened through serendipity: He was injecting more than 50 different compounds into the cortex and watching the electrical recordings unfold. When he accidentally diluted one of his compounds in the wrong chemical solution, which contained high concentrations of potassium chloride, he found that slow waves were temporarily elevated in the injected region.

Now Faraguna uses potassium chloride to strengthen synapses in discrete brain areas during wake and sleep to see how the electrical patterns of slow waves change in those brain areas. (Later, he will measure gene expression in those areas.) Toufa starts nodding off, as Faraguna watches him on a video camera feed to a computer monitor in the adjacent room. On a second computer monitor, Toufa's brain-wave patterns are unfolding in real time in two lines of red and blue. Soon the wave pattern starts looking more deliberate as the cycles become slower and larger, indicating the rat is nodding off. Faraguna points the pattern out on the screen, his voice getting softer so as not to wake Toufa.

All of this is designed to test the more theoretical part of Cirelli and Tononi's hypothesis: the connection between slow-wave activity and synaptic downscaling. Specifically, they argue that if the brain needs sleep to downscale synaptic connections, that downscaling needs slow-wave activity to occur.

So far, the only evidence they have to support this connection is indirect. People need slow-wave sleep. They also need to prune synapses. The timing of the two events also matches: Slow waves swell early in the night and taper off, while



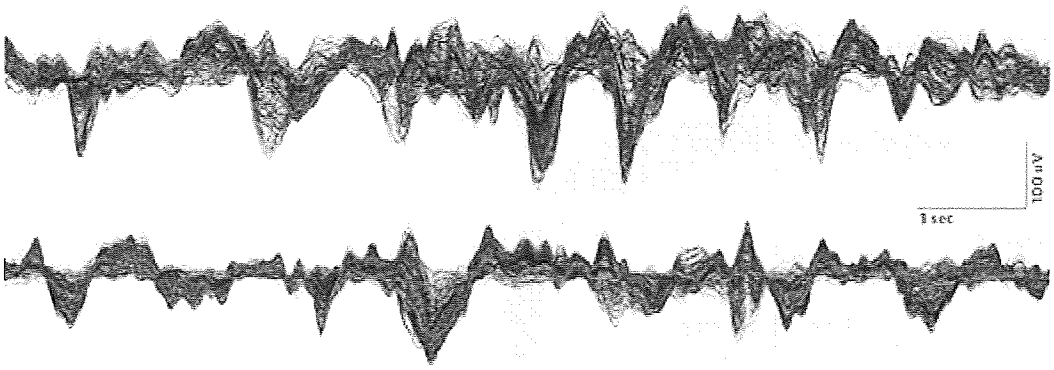
Synaptic strength (represented by "w" or synaptic weight) starts at a baseline level at the beginning of wake (bottom center). The numbers or strength of connections grow throughout the day, as animals are exposed to new situations (left), and reaches a peak level at the beginning of sleep. During the early part of sleep, slow waves are larger and more frequent (top), which theoretically could case a downscaling of synapses (right) back to baseline.

Source: Cirelli and Tononi

molecular and electrophysiological evidence in rats suggest that synapses are strongest early in the night and weakest after a long bout of sleep. This suggests that the slow waves set the process of downscaling in motion (how, Cirelli and Tononi are not sure). This part of their hypothesis is more controversial because there is no way to test whether slow waves cause downscaling, and the mechanism and function—basically the meaning—of slow waves remain unclear.

There is as yet no evidence to say with certainty that synapses are actually strengthening or weakening during slow-wave activity, Stickgold says. To establish a more direct relationship between slow waves and learning and performance (and the concomitant increase in synaptic strength), Cirelli and Tononi's graduate student Eric Landsness has worked out a way to selectively disrupt people's slow waves as they snooze in Wisconsin Sleep, a laboratory for studying human sleep located in the basement below Cirelli and Tononi's wet lab. He has spent the last two years developing a computer program that detects slow-wave patterns in the EEG as they happen, and plays a loud beep to disrupt the slow waves without waking the subject.

Normally, people trained on the motor task using a computer mouse improve



Intense (top) and less intense (bottom) slow waves. These represent signals recorded from the brain of a single human early in the night (top) and late in the night (bottom). As sleep progresses, the waves become less intense.

after a night of sleep, and the more slow-wave activity, the better the performance the next day. But in 11 out of 12 subjects deprived of slow waves, Landsness found that no improvement occurs.

When Paul Shaw, now an assistant professor of anatomy and neurobiology at Washington University in St. Louis, started postdoctoral work in Cirelli and Tononi's lab at the Neurosciences Institute in San Diego in 1997, he wanted to show that there was more to sleep than keeping synapses in check. One still October morning, while floating in the Pacific and waiting for a surfing wave, he thought of an idea he could test. He paddled back to shore and hurried to the lab before the lights switched on. He took all his flies that hadn't yet hatched, and switched one half to an environment of constant dark, leaving the rest to live in the normal light schedule. He reasoned that flies that remained in the dark, instead of seeing light at the usual time, would be introduced to a new condition and thus have increased levels of synaptic plasticity compared to flies that get exposed to their normal light routine. If the new experience of suddenly living in the dark were linked to synaptic strength, then the need for sleep (and synaptic downscaling, which Shaw didn't measure) would be higher in the dark group compared to the light. Shaw didn't think this would be true, but it was. Now looking at the effect of social experience before and after sleep, Shaw still hasn't produced evidence to refute Cirelli and Tononi's hypothesis.

For some in the sleep field, the idea that sleep is meant to downscale synapses just makes sense. "I've been in this field for over 30 years, and I think that for the first time, we have what I think is a very attractive and plausible theory as to why we sleep," says Mark Mahowald, director of the Minnesota Regional Sleep Disorders Center. "The more work they do on this, the more the theory is supported."

If this hypothesis is correct, it may prompt researchers to consider the effects of sleep and sleep deprivation on their experiments.

If the hypothesis is correct, it could inform potential treatments for sleep disorders or sleep problems associated with psychiatric disorders, some of which attempt to promote synaptic strength during wake by using electrical and chemical stimulations, Tononi says. On a more basic level, it may prompt other researchers in completely different fields to consider the effects of sleep and sleep deprivation on their experiments, says Emmanuel Mignot, a professor of psychiatry and behavioral science at Stanford University in Palo Alto. "The point is that if sleep modifies plasticity, any behavioral, in vivo memory, or drug experiment that changes sleep experimentally—drugs such as stimulants, long tasks, or tests during the daytime where animals have to stay awake—may be confounded by effects of sleep synaptic plasticity."

When it comes to sleep, memory replay and consolidation is likely not the whole story, Cirelli says. "What the [replay researchers] forget is that all the evidence of replay is for only the first 30 to 60 minutes of sleep. Then the replay goes away." That means downscaling could occur later during a stint of sleep, she says. Also, "if you think about the replay, it is not specific for sleep. It's present also in waking."

But Cirelli and Tononi's hypothesis also leaves out some observations. For example, it does not address the function of REM sleep. In contrast, the memory replay idea has, albeit indirectly, addressed REM sleep: Researchers have found signs of replay occurring during REM, and found that this stage of sleep is generally important for some types of memory.

Also, the synaptic homeostasis hypothesis focuses on synaptic changes in cortical areas, but it's still unclear whether the changes happen in other areas outside the cortex, especially areas like the basal forebrain, which is known to regulate sleep. If not a global phenomenon, synaptic downscaling might not play a central role in sleep. So far, Cirelli's work on gene expression in flies, which don't have a cortex, has shown differences in expression of synaptic plasticity markers between sleep and wake.

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But in studies of songbirds, humans, and other animals, complex changes occur in the brain during sleep that reflect learning and new experiences more than the downscaling of synapses. In 2007, Matthew Wilson, a neuroscientist at the Massachusetts Institute of Technology in Cambridge, and his colleagues found distinct neuronal spiking patterns in groups of sleeping rat cells not only in the hippocampus, long known to regulate learning and memory, but also in the visual cortex. To the sleep and memory community, this electrical trace suggests sleep is important for reactivating and

reorganizing specific memories. In contrast, Cirelli and Tononi's hypothesis leaves out the structured patterns, explaining sleep more as a global downscaling of synapses. "In order to accept [Cirelli and Tononi's hypothesis], you have to say the patterns don't matter," he says. "There is simply too much [spatial and temporal] structure to dismiss, in my opinion. The real question is—does that lead to directed changes in synapses?"

Recently, researchers led by Marcos Frank at the University of Pennsylvania in Philadelphia covered one eye in kittens, an experiment designed to strengthen neuronal connections from the visual cortex to their uncovered eye. Normally, to strengthen these connections, the kittens would need a bout of sleep. In a February study in *Neuron*, Frank and his team found elevations in neuronal firing and in several molecules critical for synaptic strengthening, such as ERK and CaMKII—but only in the animals given a chance to sleep. The findings suggest slumber strengthens connections rather than weakens them.[7]

In contrast, Cirelli and Tononi, using adult rats, have seen reductions in CaMKII and other molecular and electrophysiological signs of increased synaptic strength in the cortex after sleep.[3] Because Frank et al.'s data focus on local changes in the developing brain and the synaptic hypothesis is about the global adult brain, it's too soon to say with certainty whether the results contradict the hypothesis, Cirelli says.

"Now it may be that there [are] some differences in the type of plasticity that we're studying and what [Cirelli and Tononi are] attempting to study," Frank says. "If that's true, that means their theory can't be all encompassing," he adds. "The field as a whole will determine if these and other exceptions require a revision of [the hypothesis] or its demise."

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