### WHAT ARE THE SECRETS OF

# SPINELESS SLEEPERS?

By Zac Unger
Photographs by Céline Sadonnet

BY EAVESDROPPING ON SNOOZING JELLYFISH, RESEARCHERS BEGIN TO UNDERSTAND THE EVOLUTION AND PURPOSE OF SLEEP IN THE ANIMAL KINGDOM

**To be human is to sleep. Also, to be human is to find yourself unable to sleep and to spend endless overnight hours reading obsessively about how chronic sleep deprivation is toxic for your brain.** Every involuntary hour of wakefulness is a further strike against your cognitive function and ability to consolidate memories, regulate your emotions and repair damage to your neurons. It's almost enough to make you wish you didn't have a brain that could keep you awake reading about how much damage you're doing to your brain.

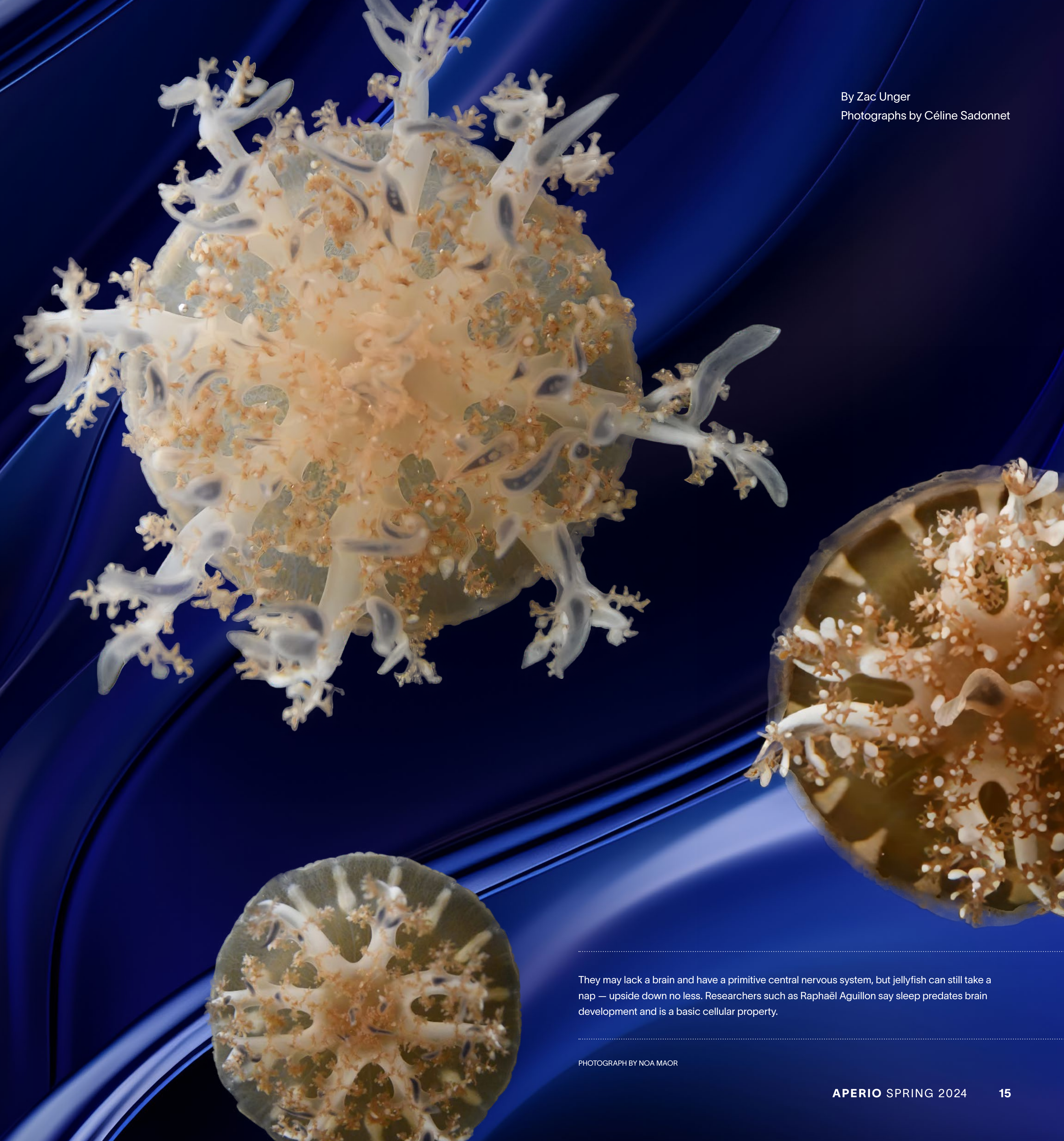
While not every animal suffers from nighttime anxiety, every species that scientists have observed experiences sleep in some fashion. By studying jellyfish and sea anemones — animals with some of the most primitive nervous systems — Raphaël Aguillon hopes to shed light on some of the fundamental questions about sleep throughout the animal kingdom.

"We have a lot of hypotheses about sleep, but there's so much that we don't know about its functions and processes," says Aguillon, who is pursuing research with an Azrieli International Postdoctoral Fellowship at Bar-Ilan University.

According to Aguillon, who refers to himself as a "marine chronobiologist," scientists have been studying sleep throughout the animal kingdom for decades. From mammals to insects, sleep has been found in each kind of organism. But there's no coherence in the quality of sleep. Some animals sleep during the day, others at night. Some sleep for long periods, others for just a couple of hours. Some sleeping animals go completely still, and some continue to move. "Whether you have a huge brain or no brain at all," Aguillon says, "you need to sleep."

His work on simple sea creatures has the potential to unravel some of the larger questions about sleep in general, such as how it evolved, what purpose it serves and why each species has different rhythms.

The universal animal need for sleep led Aguillon and his colleagues to ask an even more fundamental question: Which came first, sleep or the brain? In searching for an answer, Aguillon looked to the cnidarians (pronounced with a silent "c"), a phylum of invertebrate aquatic animals containing more than 10,000 species, recognizable to most of us as jellyfish, sea anemones and corals. These creatures look as if they're not even from the same planet. They lack a brain yet have a basic nervous system that contains both excitatory and inhibitory neurotransmitters. Neurons communicate with neurotransmitters via electrical currents — different types of neurotransmitters will either start, transmit or stop the information flowing between neurons.



They may lack a brain and have a primitive central nervous system, but jellyfish can still take a nap — upside down no less. Researchers such as Raphaël Aguillon say sleep predates brain development and is a basic cellular property.

PHOTOGRAPH BY NOA MAOR

According to research by Aguillon and his teammates in the waters of the Florida Keys and the Red Sea, these primitive neuronal structures crave sleep just like any of the "higher" animal species.

Aguillon describes sleep as having four basic characteristics: A sleeping organism must move less than a wakeful one, be less sensitive to its surroundings during sleep, sleep at roughly the same time each day, and experience sleep as an inescapable function. "You accumulate the need to sleep," says Aguillon. "You can go to a party and delay the time when you go to sleep, but at some point, you will accumulate fatigue and crash. The need to sleep will always catch up with an animal."

The basic question underlying the research is whether sleep is a function of the nervous system of an entire organism, or whether each individual cell within any animal has its own innate drive to sleep. In search of an answer, Aguillon and his lab mates focus on two cnidarians, the sea anemone *Nematostella vectensis* and a jellyfish from the genus *Cassiopea*. While the species share similar nervous systems, *Nematostella* are nocturnal whereas *Cassiopea*, like humans, are active during the day.

Unlike humans, cnidarians do not put on eye masks and shut the bedroom door to indicate that it's time to sleep, so designing a method to measure their periodicity was the researchers' first hurdle. The simplest experiment took place in Bar-Ilan's Applebaum Lab, with the subject animals placed in large water tanks. The aquaria were kept at a constant temperature with artificial lights mimicking a perfect day of 12 hours of light and 12 of darkness. With the knowledge that stillness is one of the fundamental properties of sleep — zebras lie down, dolphins float on the ocean's surface — the scientists counted the jellyfish's pulsations per minute to determine wakefulness versus sleep.

From there, the task was essentially to pester the animals in order to disrupt their sleep. For *Cassiopea* jellyfish, who like to sleep upside down, that involved targeting them with a pulse of water during sleep periods so that they would involuntarily flip over and then have to resettle themselves. For sea anemones, which are stationary and nocturnal, the scientists disturbed their sleep with powerful pulses of light.

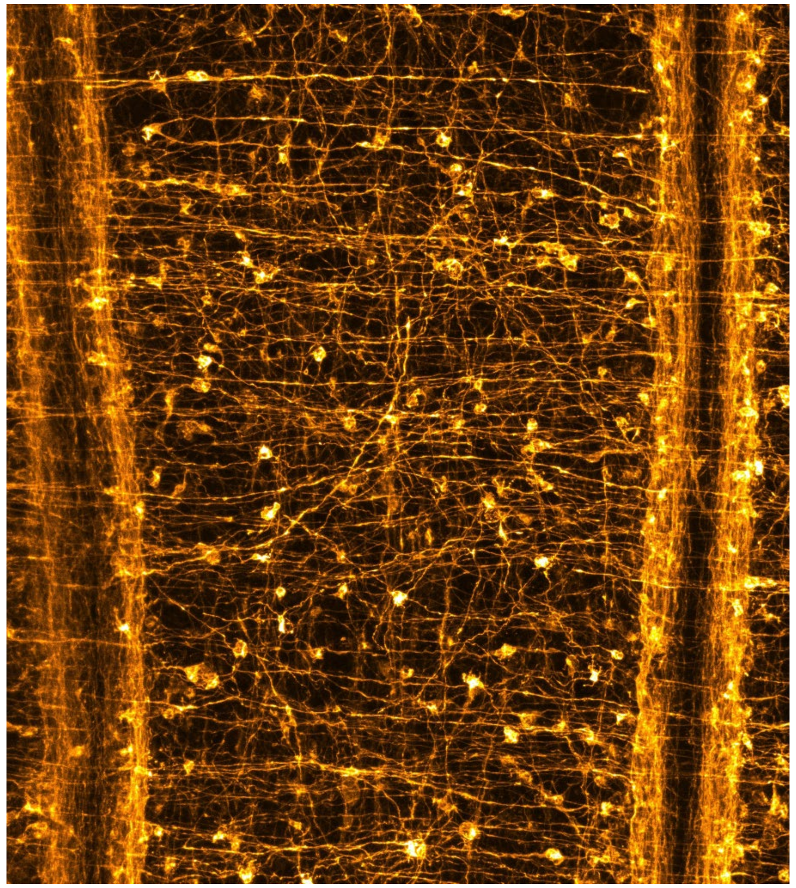
Simply keeping an animal awake doesn't tell you much about the importance of sleep, though. "Jellyfish don't have a complex cognitive life," Aguillon says, "so they probably don't need sleep to reinforce memories." Instead, simple animals, such as fish, flies and mice, express the stress of sleeplessness as damage to their DNA. Essentially, DNA begins to break down during periods of wakefulness, and is repaired during the hours of sleep. This phenomenon, he says, suggests that DNA repair was actually the first function of sleep.

In parallel with the cycles of sleep and disturbance, Aguillon and his collaborators sampled tiny bits of animal tissue that contained neurons and fixed it with chemicals to prevent degradation. Then, using microscopy, the researchers compared damage levels between periods of natural sleep and wakefulness against samples taken during bouts of enforced insomnia.

What they found was consistent with their expectations and with the experience we all have when suffering from interrupted sleep. On a cellular level, the sleep-disturbed animals exhibited higher levels of DNA damage than those that were well rested; on a behavioural level, the stressed jellyfish had to spend more time catching up on sleep during the period of the day when they would normally be awake.

PHOTOGRAPH BY NOA MAOR

PHOTOGRAPH BY RAPHAËL AGUILLON



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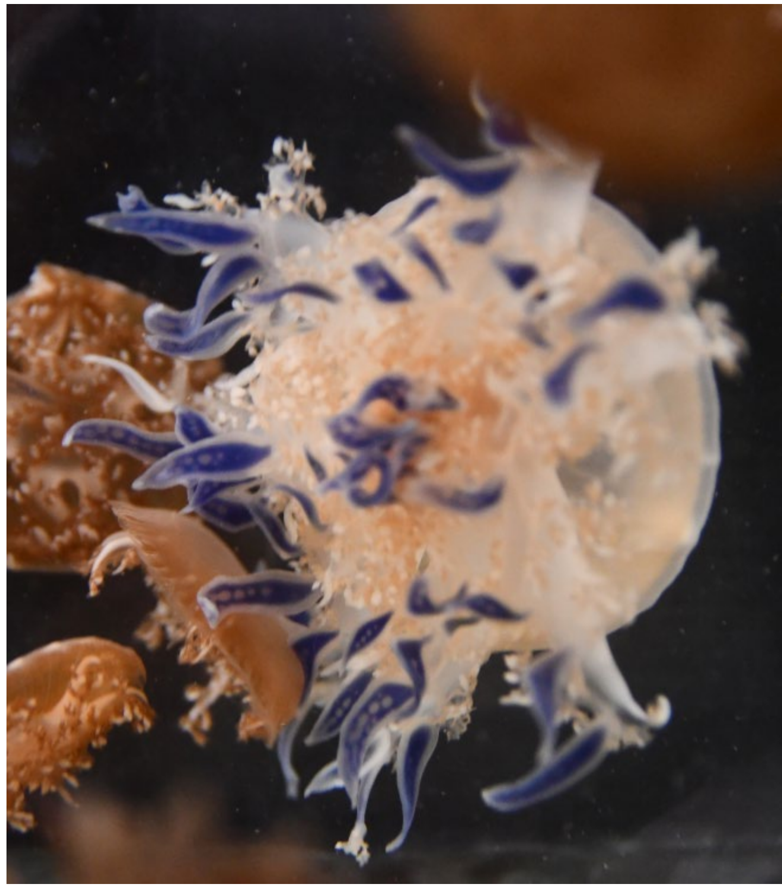


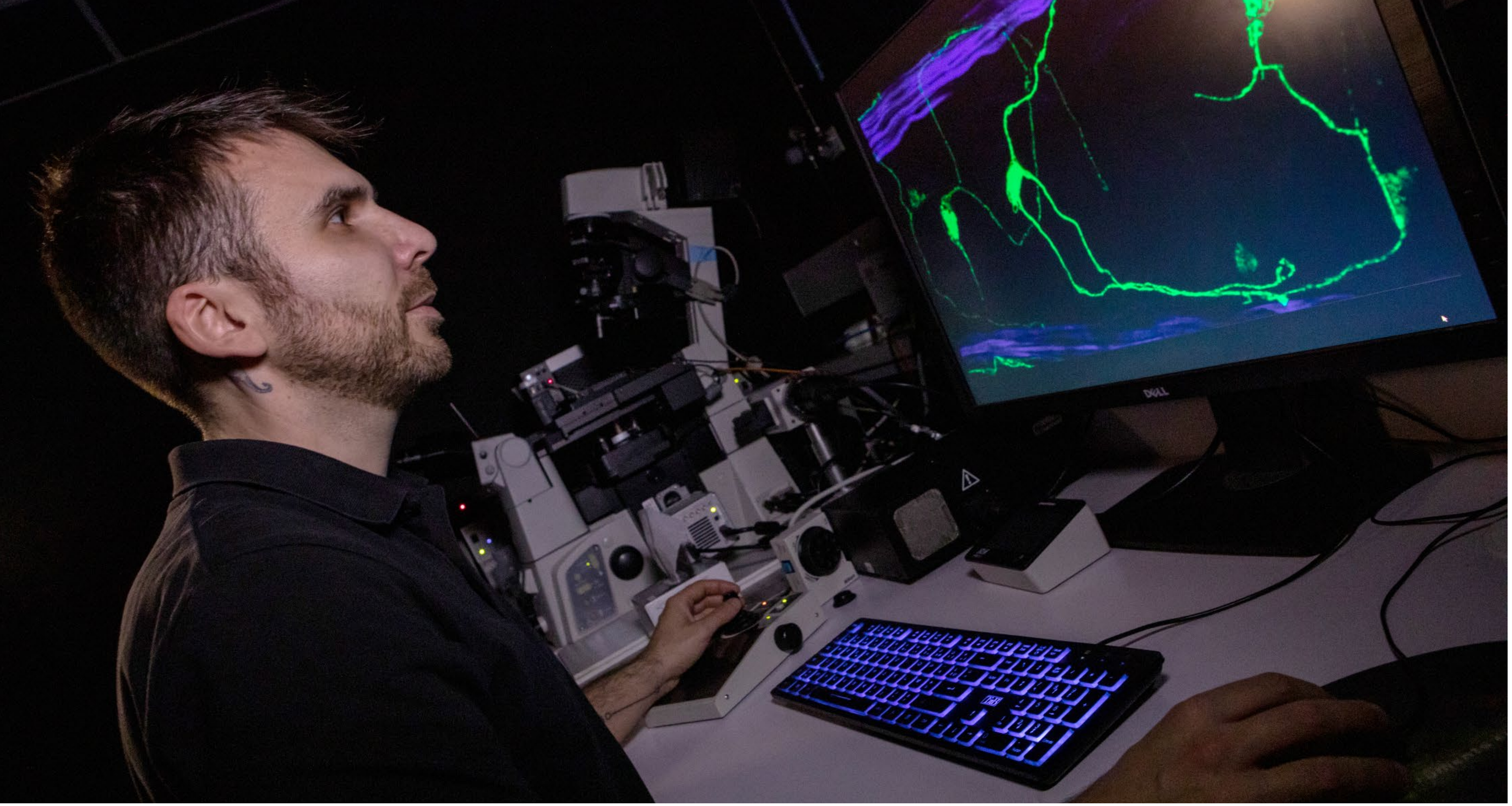
**Collaborating with labs around the globe, Aguillon acquired jellyfish that were genetically modified to be partially fluorescent, so that neurons became visible under certain light conditions**

For Aguillon (left), an Azrieli International Postdoctoral Fellow at Bar-Ilan University, a good day in the lab involves prodding jellyfish (top left and right) and studying the functional units of a sea anemone's nervous system in action (top centre).

"The results we gathered provided the first evidence of a link between sleep and the accumulation of DNA damage in animals with a diffuse neural net," says Amir Harduf, Aguillon's research partner and closest collaborator. "Moreover, animals that are active during the day will accumulate DNA damage during the day and repair it by sleeping at night," whereas nocturnal animals perfectly reverse that pattern.

Getting deeper into the analysis required more complex processes and experiments. Aguillon has a self-professed fascination with neurons, and being able to see the functional units of the nervous system in action was a key goal. Collaborating with other labs around the globe, Aguillon was able to acquire *Nematostella* that had been genetically modified to make the animal partially fluorescent, so that the neurons themselves became visible under certain light conditions. This made sampling and collection easier. And being able to see the intertwined networks of neurons made the theoretical side of the experiment more concrete and apparent. "It's not the most complex neuron system in the world," Aguillon says simply, "but it's beautiful."

Aguillon's research journey featured some unexpected turns. In his own telling, he was a bit of a listless student and was directed toward trade school as a teenager, where he spent two and a half years learning to build chairs in a particular Renaissance style. "At the end of that, they told me I wasn't so good at doing this either," he says with a laugh.



Those years of furniture making coincided with an awakening of his intellectual ambitions. He began preparing for nursing school and became obsessed with biology. After beginning his science studies at the University of Toulouse, an encounter with a professor of genetics opened his eyes to what he wanted to study. “Also, we have a lot of sailors among my ancestors,” he says, so his dual love for the sea and genetics led him to where he can often be found today, snorkel in mouth, floating motionless over jellyfish in the warm waters off the beaches of Eilat, Israel.

In Israel, Aguillon also found the kind of collaborators that pushed his study to new heights. The first year of his study was difficult, he says, but everything clicked when Harduf joined the Applebaum Lab. Aguillon’s skills in genetics and embryology paired perfectly with Harduf’s expertise in data analysis and experience with cognitive functional studies in human brains. “Everyone here is blunt, but in a productive way,” Aguillon says. “If something isn’t working, they’ll just say it. I learned that being part of a team is the best way to do this work, to collaborate and disagree and reach new conclusions.”

Opportunities to build on this teamwork for future study and practical applications abound. In particular, Aguillon is excited about using secreted sleep-deprived molecules as early indicators of ecosystem-level stress. The lights, sounds and motions of our modern world place stress on the environment with no regard to time of day or which animals might be repairing their DNA. Aguillon hopes to identify molecules released by animals undergoing periods of stress. By measuring the molecules in sea water, he says, scientists can monitor in real time or anticipate when an ecosystem is damaged. This could involve excluding boats from an area for a few days so animals can catch up on sleep or working to reduce light and noise pollution in sensitive areas.

“Sleep is a major sign in human health and a very good entry point to treat patients,” says Aguillon. “But it’s clearly uncharted territory in the ecological and environmental fields. Could using sleep as treatment work in ecosystems as well?”

Research on an organism with a neuronal structure as simple as a cnidarian’s opens a window on the very beginnings of animal evolution, says Aguillon. It teaches us that sleep is a crucial life function, a cellular property of the most basic order. “Without sleep, there would not be any nervous system,” he says. “Before the brain existed, sleep was already here.” ▲●■

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# SHEDDING LIGHT ON CIRCADIAN RHYTHMS

**One of the most crucial aspects of the sleep cycle is the circadian rhythm, the essential intuition animals have about the timing of their rest and wakefulness cycles.**

By far the most common sleep-timing cue is the sun, which repeats on a regular schedule every day. While other factors such as temperature and tide can affect the circadian rhythm, the power and predictable nature of the sun tend to make it the overriding factor.

The circadian clock acts as regulator of these different factors, anticipating external ambient patterns and helping an animal’s internal mechanisms know when to prepare for sleep or wakefulness. Even animals that live deep undersea or in caves where little light persists develop a dependable circadian clock to regulate their sleep cycles, perhaps via other cues such as food, temperature or social interaction.

People and animals placed in windowless environments with no external light cues still wake and sleep at roughly predictable times. “You have an internal representation of time,” says Aguillon. “It’s not conscious. Your body knows it.”

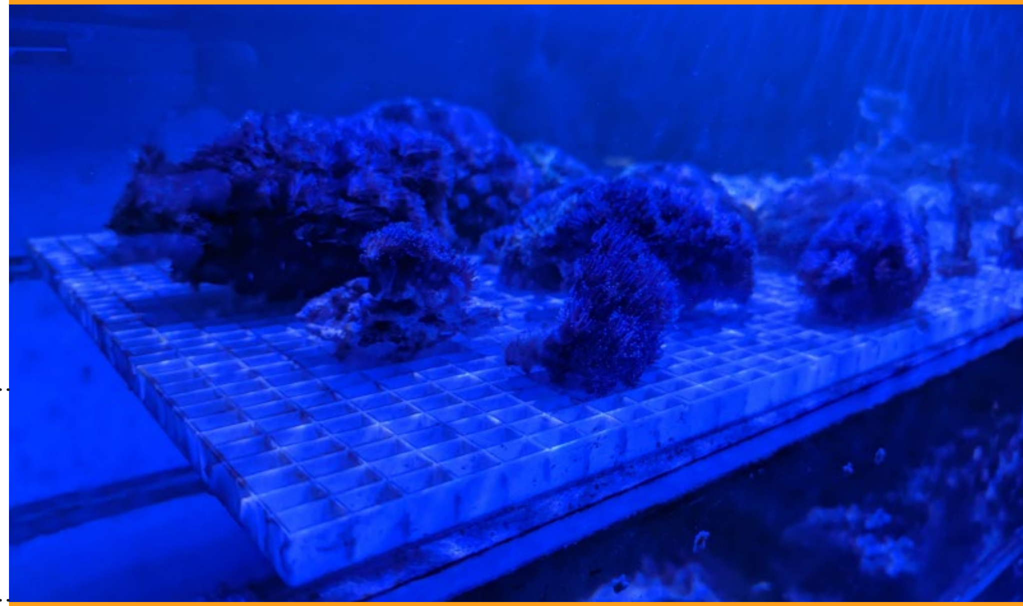
An innate organizational function this powerful is almost certainly genetic. To test the effects of sleep disturbance on these rhythms, Aguillon and colleagues identified the gene that regulates the process and then, according to Aguillon, “we broke it.”

Using the gene-editing technique known as CRISPR, the scientists isolated what they called the *Clock* gene in sea anemones, the same gene that performs similar functions in humans and other animals. What they found was an incredibly complex interaction of genetics and milieu that demands further study.

In the animals with mutated or “broken” *Clock* genes, rhythmicity essentially disappeared under conditions of constant darkness. But it also appears there is some level of cooperation between the *Clock* gene and ambient light that regulates the anemone’s circadian clock.

Dr. Mieka Rinsky, a molecular biologist who has collaborated with Aguillon in the past, says this work represents “a breakthrough in identifying and understanding the role of *CLOCK* in regulating rhythmic locomotor activity and gene expression in sea anemones.”

Future research promises to offer a clearer picture of the *Clock* gene’s role in the ancient relationship between light and the circadian rhythms. ▲●■



Is DNA repair the first function of sleep? Studying cnidarians in a controlled lab setting (right), Aguillon and his team found that animals active during the day accumulate DNA damage and repair DNA by sleeping at night. Nocturnal animals reverse the pattern.

PHOTOGRAPH BY AVIAD STOLLMAN