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A FRESH START

Fecal microbiota transplants may help desperately ill patients reclaim their lives.

PLUS

KETAMINE FOR SEVERE DEPRESSION?
SLEEPING FRUIT FLIES
FRANK NETTER'S LEGACY

Neuroscientist Nicholas Stavropoulos, PhD, studies sleep in the fruit fly using a technique called forward genetics.

An Ancient MYSTERY

SOME SAY IT'S NEEDED TO CONSOLIDATE MEMORIES OR TO CLEAR OUT METABOLIC WASTE FROM THE BRAIN. BUT NOBODY REALLY KNOWS WHY WE SLEEP. NOW, NEUROSCIENTISTS ARE PROBING THAT MYSTERY IN AN UNLIKELY CREATURE, THE FRUIT FLY.

BY JIM SCHNABEL • PHOTOGRAPHS BY BEATRICE DE GEA

BIRDS DO IT, BEES DO IT, even humble fruit flies do it—sleep.

Flies can't close their eyes, because they don't have eyelids, and their tiny brains almost certainly cannot dream. They don't snore or drool on the pillow or murmur the names of old flames in the dead of night. Yet, like pretty much every other animal with a central nervous system, they spend a large part of their lives catching Zs.

"If you deprive a fruit fly of sleep, it will sleep longer the next day, just like a human," says Nicholas Stavropoulos, PhD, assistant professor of neuroscience and physiology, and a member of the NYU Neuroscience Institute. "You can even give a fly a drug such as caffeine or methamphetamine that alters sleep in humans," he says, "and it will have more or less the same effects."

That's good news for scientists, who are always on the lookout for useful animal models of human behaviors and disorders. Of course, lab mice sleep, too, but they are comparatively slow growers, requiring a few months to develop from embryo to adult. Fruit flies—particularly the *Drosophila melanogaster* species favored by laboratory biologists—can become breeders in 10 days, making them especially well suited for genetics experiments. Individual flies measure only about an eighth of an inch long, and thus take up much less space than mice. They can live at room temperature on a simple soup of sugars and other nutrients. Dr. Stavropoulos keeps thousands of vials of *Drosophila*, each containing a line, or family, with a certain set of mutations, on racks of shelves in refrigerator-like incubators. "It's almost like a library," he says.

Of course, *Drosophila* are evolutionarily far removed from us. Yet among their four pairs of chromosomes are genes that have been largely preserved over the hundreds of millions of years since our common ancestors slithered through the Paleozoic muck. Some of these conserved genes help regulate sleep. In fact, experiments on *Drosophila* in recent decades have illuminated genes and signaling networks that maintain the daily pacemaker, the so-called circadian clock. The various parts of this key enforcer of nighttime sleep work together in surprisingly similar ways in flies and humans.

Yet much remains unknown about sleep, and experiments on flies and other animals have failed to answer the biggest question of all: Why must animals with brains—even those with simpler nervous systems such as crayfish and honeybees—revert to this inactive, almost deathlike state for at least a few hours each day?

A TWIST ON GENETIC SCREENING

A technique called forward genetics is helping biologists approach such questions. Instead of mutating one known gene to see how its loss affects a fruit fly, a DNA-altering chemical is employed to create random, unknown gene mutations in a population of flies. From this population, single flies are bred to create lines, each of which may carry a unique gene mutation. Each line can then be scrutinized for an abnormal trait of interest, such as a reduced level of sleep. If this trait is found, the genomes of these flies are screened to identify the mutation that caused it, and that gene would now be implicated in the regulation of sleep. The technique, with its potential to probe entire genomes and find genes with previously unknown functions in thousands of fly lines, is considered particularly powerful for biological discovery. "You cast a wide net. You make no assumptions," says Dr. Stavropoulos. "You embrace the unknown."

You also embrace hard work and tedium. The process of isolating and examining tiny flies for sometimes subtle abnormalities is time and labor intensive. It is also quite risky in terms of careers. Despite all that effort, the thousands of mutant fruit fly lines that one creates may not yield any of scientific interest.

Even so, Dr. Stavropoulos has been pursuing his forward genetics experiments since he earned his PhD in genetics from Harvard in 2003, hoping that he'd end up with something dramatic to show for his work. Upbeat about the possibilities of sleep research using *Drosophila,* he began a postdoctoral fellowship at The Rockefeller University and spent most of his time mutating fruit flies, breeding them into lines, and watching for abnormalities in their sleep patterns. Week after week in the prime of his life, he was *watching flies sleep*. In a typical week, he'd screen 500 to 800 flies, representing more than a hundred different mutant lines.

How do you know a *Drosophila* is asleep? Its behavior changes subtly. It stops moving and becomes slower to move when startled. Experience has taught biologists that a fly may safely be considered asleep when it has failed to cross an infrared beam in a specially designed tubelike chamber, about two and a half inches long, for five minutes. (Other work has shown that sleep does alter brain activity in flies.)

In the Rockefeller lab, Dr. Stavropoulos kept track of automated recorders that marked the intervals of fruit fly slumber. Weeks and then months went by. Some flies displayed interestingly odd patterns of sleep, but most behaviors turned out to be random quirks of individual flies that were not seen in others of their line. In two lines, the flies displayed genuine, reproducible reductions in sleep, but these flies turned out to have mutations in a sleep-related gene that others had already discovered and published in the scientific literature. He wanted to discover something novel.

By the time seven months had passed, Dr. Stavropoulos had bred and screened an eye-popping 21,000 flies, representing more than 3,500 distinct mutant lines. At this point, he was tired, but he wasn't entirely empty-handed: several lines displayed odd sleep behaviors caused by mutations in genes that hadn't been previously linked to sleep.

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One of these lines really stuck out. The flies in this otherwise ordinary family slept only about a third as many hours as usual—the equivalent of about two and a half hours per night for a human. It can take a scientist a long time to pull a true discovery out of the usual experimental tangle of bias, error, and random variation. "But this was so far outside the norm of *Drosophila* sleep behavior that I knew I had something," Dr. Stavropoulos recalls.

Nevertheless, it took him two more years to identify the fruit fly gene whose mutation had caused this weird, stay-up-all-night phenotype, and then to confirm the discovery by restoring the regular version of that gene to the mutant flies, who thereafter slept normally. In keeping with the practice of fly geneticists, he named the gene for what the fly is without it: *insomniac*.

THE MYSTERY OF SLEEP HOMEOSTASIS

The discovery led to other big questions. First, does *insomniac* have relevance to human biology or merely to fly biology? In subsequent experiments, Dr. Stavropoulos found that the fly gene does have close cousins in the genomes of humans, mice, and other vertebrates, suggesting that its function is fundamental enough to have been preserved over the eons.

Another question was how *insomniac* affects sleep. Dr. Stavropoulos found that it doesn't work through the circadian clock; it doesn't switch on at night and off in the morning as a circadian enforcer of sleep would. To a scientist seeking new and deep insights, this was good news. The basic network of genes that regulate the circadian clock

had already been largely illuminated, thanks to pioneering experiments on *Drosophila* by a number of investigators, including Michael Young, PhD, Dr. Stavropoulos's adviser at Rockefeller. Moreover, there was evidence that the circadian clock, while a fundamentally important phenomenon in its own right, isn't necessarily the best mechanism to study in searching for the ultimate purpose of sleep. "The circadian clock seems to be more ancient than sleep—there are bacteria and plants, for example, that have circadian rhythms," says Dr. Stavropoulos.

The other major regulator of sleep, which some researchers believe may offer better clues to sleep's deep evolutionary meaning, is a broad mechanism called sleep homeostasis, or sleep drive. This homeostatic mechanism constantly adjusts how much sleep an animal needs, independent of the circadian clock, which synchronizes our sleep to the earth's day and night cycles. The sleep homeostasis process seems to involve the dialing down of arousal circuits in the brain—circuits that can be pepped up by caffeine, for example—but just how it works is still unclear. Perhaps *insomniac* is one of the keys to solving that mystery.

A third big question about sleep is how (and why) its loss affects the health of an animal. Virtually all studies addressing this issue have linked sleeplessness to ill health and often to early death. Rats totally deprived of sleep die within weeks, for instance. Intriguingly, Dr. Stavropoulos found evidence that *insomniac*'s sleep-inducing function might be distinct from its health effects. Flies without the gene died young on average, but when Dr. Stavropoulos suppressed the gene *only in the flies' neurons*, not elsewhere, the insects lived an ordinary life span while sleeping only about half as much as normal. It was only a preliminary finding, but it pointed to the tantalizing possibility that sleep—some sleep anyway—isn't as strictly necessary as has long been assumed. "I think there's a lot of room for exploring that issue," he says.

The *insomniac* experiments were published in a paper in *Neuron* in 2011, earning Dr. Stavropoulos a Blavatnik Award for Young Scientists in 2012, a Leon Levy Neuroscience Fellowship in 2013, as well as a Sloan Foundation Fellowship grant in early 2014, shortly after he had established his own lab at NYU Langone Medical Center. "Nick's risky project was a success," says Dr. Young, his mentor at Rockefeller, who notes that *insomniac* now "appears to have a substantial role in the control of sleep duration."

Today in his lab at the NYU Neuroscience Institute, Dr. Stavropoulos and his growing team of scientists are continuing to study the gene. It is largely connect-the-dots work—tying the *insomniac* protein to other factors that operate together to make flies sleep. Several of these have been identified, and the data so far suggest that *insomniac* and its partners enforce sleep by enhancing the normal breakdown of proteins related to arousal.

How this sleep-enforcing network gets switched on and off in the brain isn't known, but Dr. Stavropoulos hopes that the answer will help solve the deep mystery of why brains need sleep at all. Is it to remodel synapses? To clear out neurons' accumulated metabolic wastes? To conserve energy? "We just keep pulling on the proverbial ball of yarn, hoping that in the fullness of time, we'll unravel it all and get the answers to those big questions," he says. "We know that sleep is there for a reason." ●

Using their forward genetics strategy, Dr. Stavropoulos's laboratory recently found fruit flies that wake near twilight, remain active into the early night, and return to sleep well before dawn. His team named the gene mutation in these flies *Dracula*. They are trying to clone the gene to understand how it might play a role in controlling when animals fall asleep.