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Mouse Studies Link Feeding-Behavior Gene with Narcolepsy

Researchers who had bred a group of mice in hopes of learning more about a brain hormone that stimulates appetite got a bit of a surprise when they saw that the rodents would suddenly collapse and fall fast asleep with no provocation. As a result, Howard Hughes Medical Institute investigator Masashi Yanagisawa and colleagues at the University of Texas Southwestern Medical Center in Dallas have an exciting new lead into the genesis of sleep and the origins of narcolepsy, a severe sleep disorder in humans.

In 1998, Yanagisawa discovered the orexins, small brain proteins and their receptors that regulate feeding behavior in mice. To probe the role that orexins play in regulating appetite, Yanagisawa and his colleagues developed a strain of knockout mice whose orexin genes do not function properly. After raising several generations of the mice, the investigators videotaped the animals as they went about their daily business, hoping to see how the genetic alteration changed their behavior.

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— Masashi Yanagisawa

After scrutinizing hundreds of videotapes of mice scampering, grooming, eating, and sleeping, Richard Chemelli, a pediatric research fellow in Yanagisawa's laboratory, began to feel discouraged because despite months of observation, the knockout mice did not seem any different from their normal, or wild-type, counterparts.

"Then we thought, wait a minute, mice are nocturnal. So watching them during the day is the equivalent of watching human behavior in the middle of the night. They're asleep," explained Yanagisawa. "So we started videotaping them in complete darkness using an infrared camcorder."

Chemelli studied nocturnal surveillance tapes of about 50 knockout mice and began to notice a bizarre and unexpected pattern of behavior. "The mice

would be running around, burrowing, grooming themselves. Then all of a sudden, like a switch flipping, they would turn over on one side. It looked almost like they were dead. Then in a little bit, boom, they'd jump up like nothing happened. Like a switch again," Yanagisawa said.

Their observations, which are described in a research article in the August 20, 1999, issue of *Cell*, led to the hypothesis that the missing orexin somehow alters the mouse sleep/wake cycle and causes a condition similar to narcolepsy. In humans, signs of narcolepsy usually begin during a person's teens or early 20s. With little or no warning while driving a car, perhaps, or interviewing for a job a narcoleptic person feels irrepressibly sleepy and quickly falls into deep sleep. Some people with narcolepsy experience vivid dreams; others describe a sense of paralysis. On occasion, narcolepsy is accompanied by catalepsy, in which a person goes limp without losing consciousness. In every case, however, the attack ends seconds to minutes after it begins. The only known triggers are sudden emotion, such as surprise, laughter, anger, or fear.

Patients can experience narcoleptic episodes several or many times a day for life, and while certain drugs can decrease the number of episodes, there is no cure. Narcolepsy affects males and females equally, and the condition tends to run in families.

Yanagisawa's team's discovery, along with a paper in the August 6, 1999, issue of *Cell* that describes research on narcoleptic doberman pinschers by researchers at Stanford University, are the first major insights into this life-altering condition that afflicts 125,000 people in the United States. They may also shed light on the control of normal patterns of sleep and wakefulness as well.

Yanagisawa said his first thought was that the knockout mice were having seizures. To investigate this, Chemelli and UT Southwestern psychiatrist Dr. Christopher Sinton fashioned tiny electroencephalograph (EEG) electrodes, cables, and harnesses to measure the brainwaves of the mice. The investigators expected that the needles of the EEG would spike wildly during an attack, signaling that the mice were having epileptic-like seizures.

The spikes never appeared. However, the animals' EEGs and electromyograms (EMGs), which measure muscle activity, were abnormal during the blackouts. "The simultaneous EEG/EMGs showed that the animals' sleep patterns were grossly disturbed in a way remarkably similar to narcoleptic patients," Yanagisawa said.

Sleep normally progresses from light to deeper stages, then to the so-called dream or REM (rapid eye movement) phase. A narcoleptic person lapses directly from wakefulness to REM sleep and back to wakefulness. This is the characteristic that Yanagisawa's team saw, sometimes a dozen or more times per mouse at night.

The finding was completely unexpected based on the HHMI team's initial studies of the orexins, which were reported in the February 20, 1998, issue of

Cell. "The orexin gene is expressed exclusively in a very deep part of the brain called the lateral hypothalamus," Yanagisawa explained. This structure has classically been implicated with the regulation of feeding behavior, so his group named the neuropeptide after the Greek word "orexis," meaning "appetite."

Yanagisawa said the biochemical link between orexin and narcolepsy is still a mystery. "But if you just think about it philosophically, it makes sense," he added. "When an animal gets hungry, it had better be alert. It would be bad from an evolutionary standpoint to be sleepy when it's time to hunt for food."