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Putting on the Brain's Brakes

Researchers know a lot about the genes that make our biological clocks tick, but little is known about how the brain's clock causes animals to busy themselves or to rest. Now, neuroscientists at Harvard Medical School have found a molecule that the brain uses to slam the brakes on daily activity at certain times, causing rest, though not necessarily sleep.

"The results help us understand how brain signals from the clock turn into changes in daily activity," said Sebastian Kraves, who performed the research while he was supported by a Howard Hughes Medical Institute (HHMI) predoctoral fellowship. Kraves, who earned his Ph.D. in June 2005, is now pursuing a new research area as a postdoctoral fellow at Harvard.

"Your brain can command activity and sleep at the same time."

— **Sebastian Kraves**

Kraves and Harvard Medical School neurobiologist Charles Weitz report their findings in the February 2006 issue of *Nature Neuroscience*. Their article was published on *Nature's* web site as an advance online publication on January 22, 2006.

From sleep to awake and back again, people cycle from hustling and bustling to watching TV to snoring. A region of the brain called the suprachiasmatic nucleus, or SCN, governs this cycling like a clock. Daily cycles of light and darkness feed into the SCN, adjusting our internal clocks to local time. The SCN then ticks away and sends out signals to other parts of the brain. These signals tell the brain not only when to sleep and wake, but also when to engage in physical activity or rest.

Sleep-wake cycles and activity levels appear to be somewhat independent of each other. "To use an extreme example, sleepwalking is the activation of a locomotor program in the absence of a wake program," said Kraves. Your brain can command activity and sleep at the same time, he said.

Although scientists understand a lot about how external signals set the clock and how the clock ticks, they know much less about how the SCN makes animals move about or rest. Researchers had inferred that the SCN sends out regular pulses of compounds into areas of the hypothalamus, the part of the brain in which the SCN resides. While screening for those largely unknown

compounds, Kraves and Weitz were surprised to see that one of them might be cardiotrophin-like cytokine (CLC). Common in other parts of the body, CLC had not previously been found in the brain.

To investigate CLC's function in the circadian activity cycles, Kraves first used biochemical tools to show that CLC messenger RNA resides in SCN cells that produce known clock proteins.

Then he wanted to determine if the SCN cells produced CLC constantly or cyclically. He measured the amount of CLC messenger RNA in the SCN cells of 40 mice over the course of 24 hours. He also measured the amount of activity the animals normally engaged in during that time. The amount of CLC messenger RNA increased during the resting portion of the rodents' cycle, peaked about three hours before the animals normally begin scurrying about, and dropped sharply in coincidence with the daily onset of activity. "The picture was coming together nicely," said Kraves. But he still needed to show that CLC could affect the animals' endogenous cycles of rest and activity.

To do this, Kraves put single hamsters in cages, each with a running wheel. At specific times each day, Kraves said, "hamsters have a serious, compelling urge to run." For about 12 straight hours every day they rest, even if they spend part of that time awake—grooming themselves, drinking, munching pellets of food. "But then they just jump on the wheel and run," Because their neurons produced CLC when the hamsters would normally be resting, Kraves hypothesized that giving them CLC when they were active would inhibit their activity.

So, Kraves injected CLC or control compounds into the hamsters' brains just when they would normally start running. The control compounds had no effect, but the animals that received CLC stopped exercising in their running wheels. They did not stop drinking or grooming, however, indicating that they weren't sick or paralyzed. The inhibition of activity lasted several hours, but the hamsters started running again before the day's activity cycle ended.

Kraves then blocked the ability of some of the hamsters' neurons to respond to CLC. He found that 14 percent of their running occurred during CLC's peak period, compared to less than two percent in animals who were responding normally to CLC. "CLC didn't affect the ticking of the clock," Kraves said, "just the time the hamsters were active."

Two other signals—transforming growth factor alpha (TGF- α) and prokineticin-2—are known to affect circadian activity levels. CLC seems to be a third one. The three compounds are produced at different times, Kraves noted, and overlap in their rise and fall could dictate different phases of rest between periods of activity, he suggested.

Understanding the molecular mechanisms that drive the cycles of activity and rest could help scientists identify the circuits that convert timing information from the brain's clock into rhythmic behavioral responses. Knowing these circuits might enable researchers to develop drugs to adjust a person's

propensity for rest or for action at different times of day.