BIOLOGICAL CLOCKS

How Jet-Lag Hormone Does Double Duty in the Brain

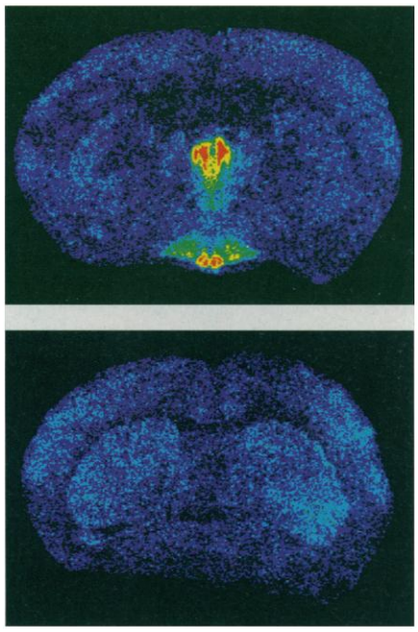
Countless travelers pop melatonin pills in an effort to get back in sync when they fly across time zones, and studies have shown that the hormone can be quite effective. But researchers have had a hard time saying why, because they understand so little of how melatonin affects the brain.

Used as a drug, the hormone is capable of resetting the brain's 24-hour circadian clock, turning it forward a bit when given at dusk and back when given at dawn. However, the shifts melatonin causes are less than an hour long—barely enough for a New Yorker to adjust to Chicago time—and that has led some researchers to expect that the key to the hormone's effect on jet lag lies in other influences it may have on the brain. A new study reported in today's issue of *Neuron* now offers some clues about what those influences might be.

By creating genetically altered mice that lack the major melatonin receptor, Steven Reppert at Harvard Medical School in Boston, Val Gibkoff at Bristol-Myers Squibb Pharmaceutical Research Institute in Wallingford, Connecticut, and their colleagues found to their surprise that this receptor is not responsible for melatonin's clock-shifting effects. Those seem to be the domain of a much rarer receptor. Instead, the major receptor underlies a second—and previously largely overlooked—function of melatonin: turning down the activity of neurons in the suprachiasmatic nucleus (SCN), the part of the brain that contains the circadian clock.

Melatonin is secreted by the pineal gland at night, at which time, Reppert suggests, its normal function may be to keep the clock from being activated and inadvertently reset by stray bursts of neural activity. Others speculate that the SCN inhibition could also account for another of melatonin's effects when taken as a drug—its ability to induce sleep, which may provide the missing link in the jet-lag mystery.

The discovery that melatonin's effects can be traced to different receptors means such speculations should be easier to test by experimentally isolating and studying melatonin's different actions on the brain, says neuroscientist Michael Menaker, who studies circadian clock evolution at the University of Virginia, Charlottesville. Circadian rhythm and sleep disorder researcher Charles Czeisler of Harvard Medical School adds that the separation of melatonin's actions also has potential value for drug development. It suggests, he says, that "analogs of melatonin could be developed that are specific to one or the other receptor," acting specifically as clock resetters or sleeping pills.



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Clean slate. Melatonin binds to slices of a normal mouse brain *(top)*, but the brains of mice missing the melatonin 1a receptor *(bottom)* show no detectable binding.

The current finding grew out of work in which Reppert's group cloned a family of genes that code for melatonin receptors. Mice have two of the genes, known as 1a and 1b, but because nearly 100% of the detectable melatonin receptors in the brain are of the 1a type, Reppert's team expected that they would carry out essentially all of melatonin's effects.

But that's not what the researchers found when they knocked out the gene for the 1a receptor in mice. To see how the lack of the receptor would affect melatonin's phase-shifting ability, Chen Liu, a postdoc in Reppert's lab, used a so-called "clock-in-a-dish" method, in which he removed the SCNs from mice and kept them alive in laboratory dishes. Work by several other teams had shown that the clock continues to run for several days under these conditions: The activity of SCN neurons rises during the day and falls at night just as it does in the intact animal. In addition, the clock in a dish responds to melatonin: The hormone suppresses SCN activity, and it sets the clock forward or back when given at dusk or dawn.

The surprise came when the researchers looked for that phase shift in SCNs removed from the knockout mice. "We got a very potent phase-shift response," says Reppert. That was unexpected, he says, because "99.9% of the melatonin receptors are gone." Preliminary evidence suggests that the 1b receptor, although so scarce as to be nearly undetectable, may be responsible for the phase shift. For example, the team found that pertussis toxin, which inhibits both 1a and 1b receptors, blocks the effect. To be sure of the 1b receptor's role, the Reppert team plans to create and study mice lacking the 1b receptor gene.

While melatonin could still shift the clock in the SCNs from the knockout mice, it totally lost its ability to inhibit SCN activity, suggesting, says Reppert, that SCN inhibition is the main melatonin action mediated by these receptors. "That effect is a bona fide action of melatonin," says Reppert, "and we have to think more seriously about what that means for the biology of the animal." There are a lot of things that can reset the clock, such as the neural activity generated if an animal is physically active at an unusual time. Reppert suggests that melatonin may act to quiet the SCN at night, "providing a level of security" by preventing such bits of neural activity from resetting the clock. Light, the most powerful phase shifter, would be the one exception, he says, because it turns off melatonin production.

That general quieting of the SCN by melatonin could explain why the hormone can relieve jet lag when it is given as a drug, says melatonin researcher Vincent Cassone of Texas A&M University in College Station. Melatonin's phase shifting alone cannot explain the effect, Cassone argues, and so the key to jet-lag relief may instead be its sleep-inducing effects. Those in turn may be rooted in its ability to hush the SCN. "The output of the SCN potently drives waking at certain points," agrees Harvard's Czeisler. By inhibiting the SCN, melatonin taken as a drug may be "tempering that drive for wakefulness." If future studies in whole animals can verify hypotheses like these, then the way is open to design drugs to optimize those effects.

But until researchers sort out the many remaining mysteries about melatonin and its receptors, they advise jet-lagged travelers to lay off the melatonin pills. Too much melatonin could "numb" the SCN, Reppert warns. The hormone also has potent effects on the reproductive system of many mammals, especially seasonal breeders like hamsters and deer. Even though humans aren't seasonal breeders, melatonin may have as yet unknown reproductive effects in humans as well. "All this ignorance," says Menaker, "makes it doubly important that people don't take melatonin indiscriminately."

–Marcia Barinaga