PHYSIOLOGY

# A 24-Hour Circadian Clock Is Found in the Mammalian Retina

Researchers have long been puzzled by an apparent mammalian deficiency. All other vertebrates have two or even three circadian clocks—the biological timepieces that govern 24-hour hormonal rhythms, sleep cycles, and many behaviors—but mammals seemed to make do with only one: a brain structure called the suprachiasmatic nucleus (SCN). But mammals may not be biochronometrically challenged after all, as Gianluca Tosini and Michael Menaker, neurobiologists at the University of Virginia, Charlottesville, report on page 419. Like other vertebrates, it seems, mammals have a second timepiece in the retina of the eye.

The retinal clock, which may govern the daily shedding and replacement of the eye's photoreceptors, seems to tick independently of the SCN clock. That means researchers trying to unravel the molecules and genes of circadian clocks in mammals now have two examples to work from. "Many of us are chasing down clock genes; this gives us another source," explains Steven M. Reppert, a neurobiologist at Massachusetts General Hospital and Harvard Medical School. Indeed, the researchers have already found that the two clocks have at least one molecular component in common, suggesting that it is fundamental to clock design.

What's more, the retinal clock should give researchers trying to tease apart the workings of these clocks a valuable experimental model. Tosini and Menaker discovered this new clock by devising a technique for keeping a mammalian retina—specifically, a hamster's—alive in culture, something never done before. There it remains sensitive to light, so that scientists can now study in culture how light enters these chronometers and synchronizes them. "It's a wonderful model and should enable researchers to nail down the nature of the clock," says Theodore P. Williams, a biophysicist at Florida State University in Tallahassee.

The hormone melatonin provided one of the first clues to the new clock's existence. The SCN was known to regulate the rhythmic production of melatonin from the pineal gland. At night, melatonin levels rise, while during the day, they fall, a cycle that may regulate daily body rhythms, such as waking and sleeping. The eye, too, has daily rhythms, renewing the tips of its rods, photoreceptors used for night vision, at the end of each night and the tips of the cones—used for color vision—at the end of each day. And because melatonin is found in the eye, researchers have long suspected that the hormone somehow paces these cycles.

But there were hints that the eye's melatonin, unlike the hormone elsewhere in the body, did not come from the pineal gland. In 1991, for example, psychologist Michael Terman of Columbia-Presbyterian Medical Center showed that a rat's daily retinal cycles persisted even after its SCN was removed. That experiment and others suggested that the retina itself was making melatonin—and in a rhythmic, clocklike manner, just as the pineal gland does. But in the absence of direct evidence of a retinal clock in mammals, some scientists, including Reppert, remained skeptical.

To show that a mammal's eye has a clock separate from the SCN, Tosini and Menaker carried Terman's research one step further by actually removing a mammal's retina and measuring the amounts of melatonin it produced. But that required keeping the retina alive for several days, a tricky thing to do because mammalian retinal tissue has a high metabolic rate. In the past, scientists found that such tissues quickly die when placed in culture because they need a copious supply of oxygen and an efficient means for getting rid of toxic wastes. To get around this problem, the team decided to culture retinal tissue at a low temperature—27 degrees Celsius—which would slow the cells' metabolism.


**Watching the clock.** Gianluca Tosini and Michael Menaker *(right)* with a *tau* mutant hamster, which has fast circadian clocks.

It was a gamble, because neurons, the kind of cell making up the retina, usually fare badly at low temperatures. "It's absolutely heretical! No one uses 27 degrees for culturing mammalian neurons," exclaims Martin Zatz, a neurobiologist at the National Institute of Mental Health. Tosini and Menaker's answer was to use the retinas of golden hamsters, which are hibernators. "That means their tissues are preadapted for survival at a low temperature," explains Menaker. And that strategy worked, Zatz notes. "That's what makes this research so neat," he says. "They found the clock with a technique that everyone knew shouldn't work."

The cooled retinas stayed alive for up to 4 days. During that period, Tosini and Menaker collected the culture medium every 3 hours, then tested it with radioactively labeled antibodies against melatonin. The retinal cells produced the hormone on a 24-hour cycle that peaked at night, proving that the clock exists and ticks independently of the SCN.

The two clocks share some basic components, however, as the researchers learned when they cultured the retina from a golden hamster known to have a mutation in a gene called *tau*. The mutation speeds up the operation of the SCN clock, giving the hamsters a shortened circadian rhythm of 20 rather than 24 hours. The cultured retinal clocks ran fast as well, implying that whatever mechanism is affected by the *tau* gene plays the same role in both kinds of clocks. "The fact that it affects a totally different clock suggests that it must be affecting something basic to clock function," says Zatz.

That's just the first of many results Menaker and Tosini expect from the retinal clock. Researchers have been able to culture the SCN, but they can't manipulate the SCN clock by changing the light-dark cycle. The test-tube retinas, in contrast, can be reset, or "entrained," with light, which should help scientists home in on the light-absorbing molecules underlying the circadian clocks' rhythms. And compared to the SCN, the anatomy and genetics of the retina are familiar ground, which should also speed the search for clock components, notes Charles J. Weitz, a molecular biologist at Harvard Medical School, who works on the genetics of the SCN. "This has real practical implications; it's a much easier place to look," he says.

It may also be a place to look for insight into certain eye diseases, such as retinitis pigmentosa, in which the rods of the photoreceptors degenerate. Because melatonin is suspected to govern the daily replacement of these rods, Menaker suggests that the disease may somehow involve a faulty retinal clock. And if unusual melatonin rhythms can harm the eye, say other researchers, it may not be wise to take melatonin supplements—the latest fad among the jet-lag set. "We'd be concerned about unforeseen consequences these supplements may have on retinal function," says Al Lewy, a psychiatrist and melatonin specialist at Oregon Health Sciences University in Portland. But now that the new retinal clock is in hand, settling such questions is only a matter of time.

**–Virginia Morell**