CIRCADIAN CLOCK

How the Brain's Clock Gets Daily Enlightenment

Researchers have discovered a new class of light-sensing cells in the mammalian retina that send their information to the circadian clock

Like a watch that runs a bit fast or slow, an animal's internal clock must be reset every day. The circadian clock regulates 24-hour patterns of behavior and physiology; it controls body temperature, schedules sleep and activity, and protests when we cross too many time zones at once.

Daylight sets this clock, and researchers have long known that in mammals, circadian photoreceptors—the neurons that detect light and send its signal to the clock—are located in the eyes. But the identities of the photoreceptors and the photopigment chemical within them that reacts to light have remained elusive.

In a burst of papers published in the past 2 months, culminating with two in this week's issue of *Science*, researchers report their discovery of a new class of light-detecting retinal cells that send their signals to the brain's clock; the cells also contain a molecule that may be the long-sought circadian photopigment.

"This is spectacular," says clock researcher Joseph Takahashi of Northwestern University in Evanston, Illinois. "It is the biggest break yet in the question of what is the photoreceptor in mammals."

The papers, from five different labs, mesh seamlessly, says clock researcher Martin Zatz of the National Institute of Mental Health in Bethesda, Maryland. "They've gone at it from a molecular perspective, from an anatomical perspective, from functional, electrophysiological, and immunocytochemical perspectives," he says. "It all fits."

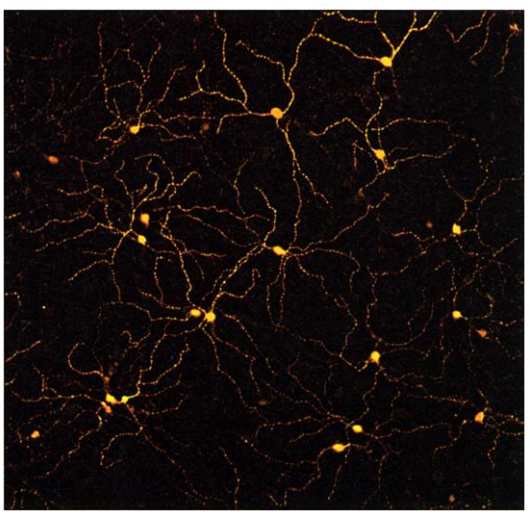
Indeed, Zatz adds, the new work describes an entirely independent light-detection system in the mammalian eye. It is intermingled with the neurons that serve vision but has unique characteristics. These are suited for detecting the level of illumination, known as luminance or irradiance, rather than the contrasts and details in which the image-forming visual system specializes. What's more, this system appears to send its signals not only to the clock, but also directly to other brain areas that use irradiance information, such as the area that controls light-activated pupil constriction.

"It looks like there is a generalized irradiance-detecting system that is regulating a variety of different non–image-forming responses to light," says photopigment researcher Russell Foster of the Imperial College of Science, Technology, and Medicine in London.

The impact of this light-sensing system may go far beyond pupil size and the clock. In humans, light levels can modulate mood and performance. "This photoreceptor system may be incredibly important in our general physiology and well-being," says Foster.

The eyes have it

Unlike other vertebrates, which have light sensors in multiple tissues, mammals detect light only with their eyes. Researchers initially assumed that mammals rely on the retina's visual photoreceptor cells—the rods and cones—for all their light sensing. But in 1999, Foster's team showed that mutant mice lacking all rods and cones still have light-responsive clocks. Some other cells in the eye had to be sensing light, perhaps using a unique photopigment to do so. Indeed, the photopigments in the rods and cones are optimally activated by light wavelengths of 500 and 506 nanometers, respectively, whereas recent unpublished results from Foster's group show a different optimal wavelength for resetting the clock of the mice that lack rods and cones.



Photopigment contender. These mouse retinal ganglion cells contain melanopsin.

CREDIT: ANA CASTRUCCI AND I. PROVENCIO

In 1998, a protein found in the eye and called cryptochrome briefly looked like a hot candidate for the circadian photopigment. But while attention was focused on cryptochrome, another molecule, melanopsin, quietly entered the scene.

Ignacio Provencio, Mark Rollag, and Guisen Jiang of the Uniformed Services University of the Health Sciences in Bethesda, Maryland, had set out to clone the photopigment in frog melanophores, skin cells that redistribute their stores of light-absorbing melanin in response to light. Their effort yielded a previously unknown member of a class of proteins called opsins, many of which act as photopigments. Provencio dubbed the protein melanopsin. And, although it hadn't been proven to respond to light, its presence in the melanophores suggested it was their light sensor.

The team went on to find melanopsin in the frog's retina as well, and that was when "a light bulb went off," says Provencio. If melanopsin were in mammalian retinas, it might be the circadian photopigment. He and his colleagues cloned the mouse melanopsin gene and, as they reported in the *Journal of Neuroscience* in January 2000, found melanopsin in the mouse retina, in a small subset of retinal ganglion cells (RGCs).

RGCs are well known to vision researchers not as light-sensing cells, but as the neurons that receive signals from rods and cones and pass them to the brain via the optic nerve. Most RGCs send their long connecting axons to brain areas involved in vision. But a few, about 1% or 2% of those in a rodent's retina, go to other parts of the brain, including the suprachiasmatic nucleus (SCN)—the home of the circadian clock.

Clifford Saper of Harvard Medical School in Boston runs a lab that studies the RGC-SCN link. Last May, Josh Gooley, a first-year graduate student, approached Saper with an idea for a rotation project: He wanted to see if the RGCs that contain melanopsin connect to the SCN.

Gooley injected the SCN of rats with a dye that marks neurons by traveling backward along their axons to their cell bodies, thus labeling the small fraction of RGCs that link to the circadian clock. He then stained the retinas with a DNA probe for melanopsin RNA. By September, Gooley had his answer: It marked the very same cells.

Meanwhile, members of Jens Hannibal's team at the University of Copenhagen took a different path to the same result. They had shown that RGCs that link to the SCN contain a peptide called PACAP. When they double-stained for both PACAP and melanopsin, they found melanopsin in the PACAP-containing cells.

The Saper group published its work in December 2001 in *Nature Neuroscience*; the Hannibal team, in the online January 2002 issue of the *Journal of Neuroscience*.

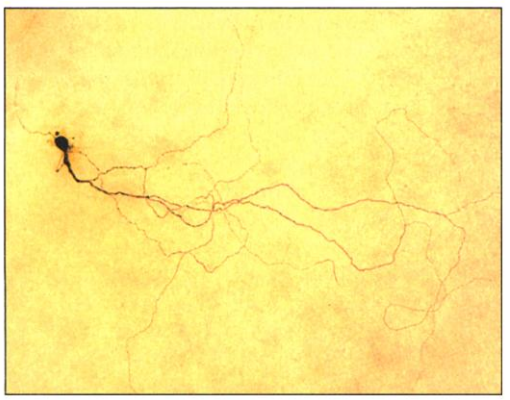
The two papers confirm what many suspected when they saw Provencio's paper 2 years ago: The neurons that connect to the clock contain melanopsin.

Lighting the path

Neurophysiologist David Berson of Brown University in Providence, Rhode Island, didn't need to hear that before making his move. Provencio's paper, Berson says, made it "absolutely plain" that these RGCs were "the prime candidate for the missing photoreceptor." Normally, RGCs are activated indirectly by light signals from the rods and cones. It would be "completely revolutionary" for them to respond directly to light, Berson says. "I just had to find out if they did."

Berson was well positioned to do that. His team specializes in recording the electrical activity of RGCs. The researchers injected a dye into the SCNs of rats to label the RGCs that connect there, then they removed the rats' retinas and recorded the electrical responses of the labeled RGCs.

"We knew we were onto something" right away, Berson says, because the cells fired in response to light. Clearly, they weren't being triggered indirectly by the rods and cones, because in Berson's preparation, the rods and cones are incapacitated. That meant the RGCs must be responding directly to the light.



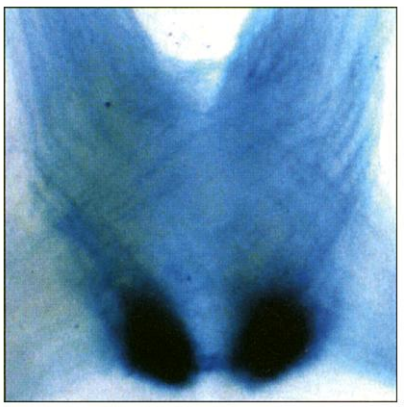
Seen the light. This rat retinal ganglion cell fires in response to light.

To confirm this, Berson's team added drugs to block all neuron-to-neuron signaling in the retinas, making it impossible for the RGCs to be activated by other neurons. When light hit the retinas, the cells still fired.

For a final check, the researchers removed individual RGCs from the rat retinas, and the isolated cells responded to light. Those results, published on page 1070, "are definitive," says Zatz. "They clearly demonstrate that this subset of ganglion cells is directly photosensitive."

Researchers had now shown that melanopsin is in a small set of RGCs that connect to the clock, and that RGCs linked to the clock respond directly to light. To close the circle and show that the melanopsin-containing and light-sensitive cells are one and the same, Berson and his postdoc Motoharu Takao teamed up with King-Wai Yau of Johns Hopkins University in Baltimore and his student Hsi-Wen Liao, who had made antibodies to melanopsin.

Takao labeled the RGCs that send axons to the SCN and confirmed that they were light responsive, then Liao used the antibodies to show that those same cells contain melanopsin. That finding suggests that melanopsin is the photopigment that allows some RGCs to register light, a signal those RGCs then send to the circadian clock.



Light messenger. Blue-stained axons of melanopsin-containing retinal ganglion cells reach the suprachiasmatic nucleus (dark blue) via the optic nerve.

Beyond the clock

In their paper, which appears on page 1065, Yau and colleagues report another intriguing result. Using mutant mice engineered by postdoc Samer Hattar, they traced the axons of the melanopsin-containing RGCs and found that they don't all go to the SCN. Some connect to other brain areas involved in circadian function or the part of the brain that constricts the pupils in response to light. The neurons seem to communicate with still other regions that remain to be studied closely. "It looks to be a single population of ganglion cells, namely, those that contain melanopsin, that go to all these different targets," Yau says.

Apparently, the light-sensitive RGCs make their information about luminance levels available to multiple brain systems that can use it. Pupil constriction is one, and Berson has evidence suggesting that the RGCs contribute to that system: His team found that the best wavelength of light for activating the melanopsin-containing RGCs is the same as the optimal wavelength that Foster and his colleagues Robert Lucas and Ronald Douglas reported for triggering pupil constriction in mice without rods and cones.

These special RGCs are well suited to the job of detecting irradiance, a mission quite different from that of the rods and cones. Rods and cones relay details about the light coming from individual points in the visual scene; to do this, each rod or cone cell responds only to light shone on a very small spot. But the RGCs Berson studied respond to light striking a broad expanse of the retina.

That complements what Provencio and his team found when they stained RGCs for melanopsin. They reported in the 31 January issue of *Nature* that the melanopsin-containing RGCs have large melanopsin-filled networks of receptive endings, called dendrites, that cover large patches of retina. "This looks like a net that is spread out through the whole retina to catch photons," says clock researcher Michael Menaker of the University of Virginia in Charlottesville.

Berson and his colleagues found other ways in which the light-responsive RGCs are particularly tuned to detect luminance. The neurons are not as sensitive to light as the rods and cones are, and they do not change their sensitivity in response to changes in luminance. Rods and cones need to turn down their sensitivity when the ambient light brightens suddenly, for example, or we would be blinded.

The light-responsive RGCs are slow to react to luminance changes but then respond continuously without adapting for at least 20 minutes. That ensures a stable reading of average light levels—just what the circadian system needs, says Provencio, to weed out "photic noise" and prevent the clock from being shifted, for example, by a brief flash of lightning. "The properties of this particular subset of retinal ganglion cells are just perfect for the job," says Menaker.

Although researchers generally agree now that some RGCs are photoreceptors, they are reluctant to bestow the title of circadian photopigment on melanopsin just yet. "Melanopsin is clearly the best candidate we have, because it is turning up in the right place," Foster says. But unlike other opsin molecules, melanopsin has still not been proven to react to light.

The gold standard for proving that a molecule is a photopigment is to make a lot of the protein in cultured cells, reconstitute the protein in its natural state, shine light of various wavelengths on it, and measure its absorbency. Researchers have done this with the opsins from rods and cones and other closely related opsins. But it's tricky to get the protein to fold correctly, and researchers have not been able to achieve this with either cryptochrome or melanopsin.

Until melanopsin is shown to be a photopigment, says Foster, researchers must consider that there may be some other photopigment in the RGCs that project to the SCN. Some still view cryptochrome, which is found in some RGCs, as a candidate. Saper's team at Harvard is using cryptochrome probes from Aziz Sancar of the University of North Carolina, Chapel Hill, to see if the cryptochrome-

CREDITS: (TOP TO BOTTOM) S. HATTAR AND K.-W. YAU; FELICE DUNN AND D. BERSON

containing RGCs link to the SCN.

Meanwhile, those studying melanopsin will soon have another tool: mutant mice that lack the pigment. "I think there is a high probability they are going to see some defect" in those mice, says Northwestern's Takahashi. A defect would be further evidence that melanopsin has a light-sensing role.

But it may be too much to expect the clocks in the mutant mice to be totally unresponsive to light. The circadian clock is too important for survival to rely on just one photopigment, Takahashi says. Although melanopsin may be the clock's main light detector, most researchers expect that there are other sources of light information—perhaps from the rods and cones, or from cryptochrome-containing cells—that may fill in if melanopsin is knocked out.

"It is very satisfying that everything has come together this way," says clock researcher Greg Cahill of the University of Houston about the recent progress. After years of learning which cells and molecules are not the circadian photoreceptors, he says, "now, we have really nice information about what they might be." **–MARCIA BARINAGA**

MEETING PRIMATOLOGY

Homeland Defense In the Wild

INUYAMA, JAPAN—The 100 participants in "Research on Long-Lived Animals" held here 15 to 18 January discussed the driving force behind male territoriality in chimpanzees, the behavior of gibbons toward long-lost relatives in need, and a successful effort to protect muriquis in Brazil. But they also explored the sociability of primates, with both welcoming and farewell parties and a trip to a traditional Japanese pub.

Territorial Motives

Are male chimpanzees after food or sexual favors when they seek to expand their territory? An analysis of 25 years of data from the Kasakela chimpanzee community at Gombe National Park in Tanzania suggests that both reasons apply, with nutrition taking the lead in this long-running debate among primatologists.

The Kasakela community may be the best studied group of primates on Earth thanks to the pioneering work of primatologist Jane Goodall in the early 1960s. Although Goodall is still involved, most of her efforts now go into conservation. Local workers have continued tracking individual animals from nest to nest, recording their movements, diet, weight, companions, and interactions, with both group members and neighboring communities. But their notebooks were accumulating dust in Goodall's house in Dar es Salaam until 1990, when Anne Pusey and colleagues at the department of ecology, evolution, and behavior at the University of Minnesota, Twin Cities, shipped them to the university's Jane Goodall Institute.

Using computers to tease out a number of subtle changes over time, Pusey presented evidence here that male territoriality is aimed at gaining access to food rather than attracting mates, as many believed. At the same time, a copious food supply has the added benefit of enticing more females. "This is really something of a merger of the two theories," says Pusey. A report on a portion of these results is in press at *Animal Behavior*.

Chimpanzee communities consist of roughly equal numbers of adult males and females and their offspring. Males stay with the natal group for life. Interactions with male chimps from neighboring groups are always hostile, usually fiercely so. Juvenile females typically migrate to a different group. Once they become adults, however, females usually remain with a community and tend to stay near the center of the community's territory.

CREDIT: A. PUSEY

Banana bait. A treat lures Gombe chimps onto scales to be weighed.

Pusey's analysis refines that observation by showing that adult females modified their roaming pattern in accordance with changes in the community's boundaries, which varied over time from 5.5 to 13 square kilometers. When the group's territory grew, the core area covered by adult females also expanded. When the group's territory shrank, adult females restricted their movements to a smaller core to avoid being at the edges of the territory.

When the Kasakela group expanded its territory, males were not rewarded with more females, Pusey says, presumably because females in the neighboring community also restricted their movements to a smaller core area as their group's territory shrank. Indeed, when adult females did occasionally wander into the fringes of Kasakela territory, Kasakela males drove them away. "So we don't think access to females is the prime motivating factor in defending territory," Pusey concludes.

Instead, the analysis supports the hypothesis that males defend a feeding range for themselves, resident females, and their offspring. Signs of better nutrition followed an increase in the group's territory: Average body weight climbed, resident females were more likely to show the swelling of the rump indicating fertility, and their babies were born closer together.

Even so, an expanding empire may eventually pay off in the mating game. Pusey's team also found that juvenile females were more likely to join the Kasakela community when its territory was at its maximum. Perhaps they recognized that the group commanded a good food supply, Pusey speculates, or were attracted to heavier, better fed males. "One of the benefits of long-term studies is that you can answer questions you might not even have thought of when you started the study," says Jan van Hooff, a primatologist at the University of Utrecht, the Netherlands. "And these results from Gombe are a good example."

Why the size of a community's territory waxes and wanes so much remains a puzzle, however. Pusey says recent studies of chimp groups adjacent to the Kasakela community suggest that groups expand their range when they outnumber their neighbors. But more long-term data are needed to understand what triggers these growths and declines in population, data that might yet be found in the Gombe notebooks.

Set Out Another Plate, Ma

A crisis often brings families closer together. That adage is as true for gibbons as for humans, according to new work by Teruki Oka, a primatologist at the Forestry