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Individual cells isolated from biological clock can keep daily time, but are unreliable

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Alexis Webb enters a small room at Washington University in St. Louis with walls, floor and ceiling painted dark green, shuts the door, turns off the lights and bends over a microscope in a black box draped with black cloth. Through the microscope, she can see a single nerve cell on a glass cover slip glowing dimly.

The glow tells her the isolated nerve cell is busy keeping time.

Webb, a graduate student in the neuroscience program, working with Erik Herzog, Ph.D., associate professor of biology in Arts & Sciences; Nikhil Angelo, an undergraduate biology major; and James Huettner, Ph.D., associate professor of cell biology and physiology in the School of Medicine, has demonstrated that individual cells isolated from the biological clock can keep daily time all by themselves.

However, by themselves, they are unreliable. The neurons get out of synch and capriciously quit or start oscillating again.

The biological clock, a one-square millimeter area of the brain called the suprachiasmic nucleus, or SCN, just above the roof of the mouth and atop the crossing of the optic nerves, comprises about 20,000 neurons.

These cells, remarkably, contain the machinery to generate daily, or circadian, rhythms in gene expression and electrical activity. But the individual cells are sloppy and must communicate with one another to establish a coherent 24-hour rhythm, says Herzog.

These features make the SCN a flexible clock that can reset to stay in synch in an ever-changing environment. The underlying sloppiness is probably what allows us to adjust to local time when we cross time zones and to vary our sleep cycles with the season, say the WUSTL researchers.

The research is being published the week of Sept. 7 in the online Early Edition of the *Proceedings of the National Academy of Sciences*.

"We've known for more than 15 years that unicellular organisms like cyanobacteria can keep 24-hour time, and isolated cells from the marine snail eye can as well," says Herzog. "But nobody was sure whether individual cells in vertebrates are circadian pacemakers."

The SCN includes many kinds of neurons that make different neurochemicals and connections within the SCN and to other parts of the brain.

"Some scientists felt that all of the cells in the SCN would be intrinsically rhythmic and that there was nothing special about any of them," says Herzog. "Some thought that none of the cells would be rhythmic and that the rhythm arose instead from their network interactions, and a third group thought specialized SCN neurons would be rhythmic and the others wouldn't be at all capable. Our experiments proved all three hypotheses wrong."

Capturing the rhythm

Webb digested slices of mouse SCN with enzymes to isolate individual neurons and then plated the cells sparsely on a dish. "The neurons will actually attach to the glass and grow," says Webb. "And as long as you give them all of the nutrients they need, they'll live for months."

The cells had been genetically engineered to glow whenever they expressed the time-keeping gene Period 2. (The cells came from transgenic mice where the Period 2 gene had been linked to one found in firefly tails.)

The rhythmically waxing and waning glow was detected by a camera designed to capture the light from distant stars and so sensitive that it will register the passage of even a single cosmic ray.

The recordings showed that all cells seem to be able to keep a 24-hour rhythm — there are no special pacemaker cells — but they don't seem to do it all the time. Neurons that make different neurochemicals show circadian rhythms in gene expression, and none was more dependable than the others.

"Single cells sometimes will be very robust and rhythmic, but most of the time they quit or lose the rhythm," says Webb. "It appears that the network structure of the SCN is important for stabilizing these sloppy intrinsic rhythms."

To show that different kinds of SCN neurons did not have rigidly defined roles, Webb exposed SCN to the drug TTX, a pufferfish toxin that shuts down cell-to-cell communication. "In a sense we just isolated the nerve cells again," she says, "but chemically rather than physically and in a reversible way."

She washed off the TTX, and then added it again, to see if the second time the cells were exposed to the toxin, they would behave the same way.

"We found cells that changed their behavior," she says. "So the first time they were isolated, or uncoupled, with TTX, they continued to oscillate, but the second time they stopped oscillating. But we also saw the reverse:

cells that were non-oscillatory becoming oscillatory."

Paradoxically the sloppiness of the clock is what makes it so precise.

"The SCN is the master clock that synchronizes other biological clocks, like your liver or your lung. Those peripheral clocks can keep 24-hour time, but not for very long," says Herzog. "Because the SCN is built differently, it can self-sustain — it can keep on ticking like a good Timex."

The researchers are now focusing on the connections that help synchronize and stabilize these biological oscillators.

Source: Washington University in St. Louis (news : web)

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