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Chimeric Mice Reveal Clues to How Brain's Clock "Ticks"

By studying mice whose brains contain a composite of neurons that produce normal and longer-than-normal circadian rhythms, researchers are beginning to understand how neurons synchronize their oscillatory behavior to control the body's 24-hour, internal clock.

The experiments represent the beginning of a new research direction, say the scientists, that progresses beyond discovering the genes that produce the internal clock machinery to exploring how brain cells interact to produce coherent circadian rhythms.

The scientists also say that the technique of producing genetic composite, or "chimeric," mice offers a promising way to study how cells in different regions of the brain work together to produce specific behaviors.

Most biological clocks operate on a 24-hour, circadian (Latin for "about a day") cycle that governs functions like sleeping and waking, rest and activity, fluid balance, body temperature, cardiac output, oxygen consumption and endocrine gland secretion. In mammals, the main circadian clock components reside in cells in the suprachiasmatic nucleus (SCN) of the brain. Inside these cells, the molecular components of the clock are "rewound" daily by the effects of light and other stimuli.

In an article published in the April 6, 2001, issue of the journal *Cell*, Howard Hughes Medical Institute investigator Joseph S. Takahashi and Sharon Low-Zeddies, both at Northwestern University, reported that they created more than 200 distinct chimeric mice whose suprachiasmatic nuclei had differing ratios of normal and mutant circadian neurons.

The mice were genetically engineered using a standard technique for producing chimeric mice. The researchers combined eight-cell embryos from wild-type mice with cells from embryos that contained a mutant *Clock* gene, which produces a loss of circadian rhythms and a period length of 27-29 hours in homozygous animals (mice with two copies of the mutant gene).

These aggregate embryos usually spontaneously form a single embryo, which can then be implanted in a surrogate mouse that would give birth to a

chimeric mouse. Since the wild-type mice were albino and the mutant mice were pigmented, the scientists could determine which animals were chimeric by their variegated coat colors and eye pigmentation. Also, the *Clock*-mutant cells carried a genetic marker for a characteristic dye, so that the scientists could distinguish *Clock* cells from wild-type cells upon examining the animals' brains.

The scientists measured the chimeric animals' circadian behavior using standard analyses of the amount of time they spent on running wheels in their cages. "One of the important facts established in earlier research with rats and mice was that individual SCN neurons could generate their own circadian oscillations *in vitro*," said Takahashi. "Those experiments were important because they showed that the circadian oscillator in mammals is cell-autonomous or cell-intrinsic to SCN neurons."

A second important point learned from earlier studies, said Takahashi, was that the *Clock* mutation reduced the amplitude and lengthened the circadian rhythms of individual neurons *in vitro*. Finally, he noted, researchers had found that rats and hamsters in which the SCN had been lesioned lost circadian rhythm. Transplantation of SCN tissue restored circadian rhythms in the animals.

All of these earlier experiments suggested that studying chimeric *Clock* mice might offer new insights into how SCN cells work together to generate the animals' circadian rhythms, said Takahashi. Studying the chimeras would have a considerable advantage over studying tissue-transplanted animals because the structure of the SCN would remain intact, he said.

Low-Zeddies and Takahashi measured and analyzed the circadian activity of chimeric mice whose SCNs ranged from mostly *Clock*-mutant cells to mostly wild-type. Their studies showed that behaviorally about one-third of the chimeric mice appeared to be normal wild-type animals, one-third homozygous mutant and one-third intermediate.

"This suggested that in order to dominate the animals' behavior, the SCN had to have a majority of one cell type," said Takahashi. "That might seem obvious, but it turned out that wasn't predictable because lesion experiments showed that if you have just a few cells left in the SCN, those are sufficient to generate rhythms," he said. "But we clearly found that the SCN needs a majority of one cell type to dominate behavior."

According to Takahashi, however, one of the most interesting findings was that some of the intermediate chimeric mice behaved like genetically mutant animals that were heterozygous—that is, each of their cells contained one *Clock*-mutant gene and one wild-type gene. Both the intermediate chimeric animals and heterozygous mutants showed intermediate 25-hour circadian rhythms.

"This result argues strongly that cell-cell interactions and integration of these periods must be occurring in these mice said Takahashi. "And because the periods in such chimeras are coherent and stable, the only way to get that is for all the cells to be synchronized together."

Comparative analyses of the chimeric animals by Low-Zeddies and Takahashi revealed that the period of circadian oscillation and the amplitude of an animal's activity did not always co-vary. In contrast, in *Clock*-mutant animals, the lengthening in circadian period is always accompanied by a lowering of the amplitude of a mutant animal's activity.

"We don't believe that anyone has found that period and amplitude can vary independently," said Takahashi. "Such findings are so complex and fine-grained, it would not have been possible without such a very large number and range of animals."

Additional studies will be needed to understand the details of how SCN neurons coordinate circadian rhythm, said Takahashi. However, he said, this new strategy represents an important future direction for understanding the physiological organization of circadian rhythms.

"Over the last four years, the field has been immersed in gene discovery and description of the molecular mechanism of the circadian clock in mammals and flies and other organisms," he said. "Of course, the genes are important, but to understand the behavior of the animal, we have to understand how cells interact in the brain to produce coherent circadian behavior." Finally, Takahashi emphasized, the chimera experiments demonstrate a new role for studies of such animals.

"Chimera analysis has traditionally been applied to developmental questions in mouse biology," he said. "But this study shows that it can also be applied to study how brain structure governs behavior, which has traditionally been thought of as too complex a mechanism to study in this way."