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Convulsions in Worms Mimic Epileptic Seizures



Image Title: Guy A. Caldwell, standing, and his research team: Shelli Williams, left; Cody Locke, center; and Kim Caldwell, right. - Michael Clemmer

Researchers at the University of Alabama have found a way to mimic epileptic seizures in the tiny roundworm *C. elegans*. The finding could make the worm a powerful model for unraveling the molecular regulation of epilepsy, a condition that affects two percent of the population.

Guy A. Caldwell, coordinator of the Howard Hughes Medical Institute's (HHMI) Undergraduate Research Intern Program and assistant professor of biological sciences at the University of Alabama, led a research team that included Kim A. Caldwell, assistant professor of biological sciences and director of the university's HHMI-sponsored Rural Science Scholars Program; Shelli N. Williams, a Ph.D. student; and two HHMI undergraduate research interns, Cody J. Locke and Andrea L. Braden. They studied worms with a mutation in the *LIS1* gene. In its human form, the gene has been linked to a rare birth defect called lissencephaly, which affects one out of every 30,000 children born. In children with lissencephaly, the normally wrinkled surface of the brain's cortex is smooth. They also have mental retardation and severe epilepsy, the causes of which are not well understood.

The team traced the mutation's effect on specific neurons in the simple nervous system of the 1-millimeter roundworm and published their findings in the September 15, 2004 issue of the journal *Human Molecular Genetics*, published online August 31.

"Epilepsy is still a black box on a genetic level."

- Guy A. Caldwell

“The human brain has 100 billion neurons, whereas the worm has only 302. We know each type of neuron and how they connect to each other,” Caldwell explained. “We knew that LIS1 is highly expressed in the nervous system, so we wanted to see if there was a way to use *C. elegans* to understand and simplify the complexities of brain disease.”

The team identified a mutation in the *LIS1* gene that causes the encoded protein to be only one-fourth the length of the normal protein. The mutation was lethal to 70 percent of the mutant worms. The team tested the survivors for nervous system defects. Although their nervous systems seemed to be organized correctly, the mutant worms were more susceptible to epilepsy-like convulsions than normal worms.

To induce convulsions, the researchers fed the mutant worms pentylenetetrazole (PTZ), a chemical that interferes with the activity of the most common type of inhibitory neurotransmitter, known as gamma-aminobutyric acid (GABA). By preventing GABA from inhibiting motor neurons, PTZ causes neuronal overexcitability, resulting in convulsions.

Although the doses of PTZ used in these experiments did not cause convulsions in normal worms, when the researchers administered the chemical to the mutant worms, the lower half of their bodies remained motionless, while muscles in the upper half contracted repeatedly, so the worms appeared to be bobbing their heads.

Since the number, placement, and organization of the GABA neurons in the mutant worms appeared normal, the team decided to look at the GABA neurons' ability to release neurotransmitters. By attaching a green fluorescent tag to a protein associated with the synaptic vesicles, the sacs that transport neurotransmitter molecules, the team was able to visualize those vesicles.

They carefully examined how vesicles lined up at the ends of nerve cells to release neurotransmitters. In normal worms, these sacs arranged themselves in an orderly fashion at the synapse, but in the *LIS1*-mutant worms, the researchers noticed gaps in the synaptic vesicle line-up.

The LIS1 protein interacts with a molecular motor protein called dynein, which helps transport the neurotransmitter vesicles within cells. In further experiments, the researchers found that blocking production of dynein caused similar convulsions and gaps in vesicle distribution as the *LIS1* mutation. Caldwell's team now thinks that mutated *LIS1* might misdirect the movement of synaptic vesicles. If vesicles fail to migrate properly to the end of the cell, GABA neurons might release less neurotransmitter and therefore not fire properly. That, in turn, could lower the worms' convulsion threshold.

"Neurons are still just cells," said Caldwell. "Trafficking defects may manifest themselves in different ways in different cell types, and seizures may be one result in neurons."

Caldwell hopes that the *C. elegans* convulsion model may help decode the mysteries of epilepsy and perhaps lead to better treatments for seizures. Researchers can knock out genes rapidly in worms, treat them with a variety of chemicals, and use other genetic techniques that are not possible with human patients.

"Epilepsy—one of the worst parts of lissencephaly—is still a black box on a genetic level," Caldwell added. "This work shows that we can dissect out that part of the disease. It is one of those classic situations where a rare disorder is going to provide insights into a more common one, epilepsy."

"It's quite possible that other gene mutations might show susceptibility to convulsions in worms," said Caldwell's undergraduate co-author Locke, who, with support from HHMI and a National Science Foundation Career award, has developed a computer database of candidate epilepsy genes to test in the worm model. Named to *USA Today's* All-American Academic Team this year, Locke credits Caldwell's enthusiasm for motivating him to pursue research as an undergraduate.

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