

## CELLULAR\_BIOLOGY

# Where Nerve and Muscle Meet

**From birth, John's muscles didn't work the way they should. At a month old, he couldn't hold up his "floppy" head. At six months, his eyelids were droopy. By age nine, he needed a wheelchair to get around.**

Now 15, John — not his real name — still lives with the difficulties of his condition. His symptoms, progressive muscle weakness and fatigue, characterize a neurological disorder called slow-channel congenital myasthenic syndrome. But a series of lab tests on biopsies from his muscles revealed something puzzling.

The electrical current that triggers muscle cells to fire continued longer than it should before shutting off — the "slow-channel" response that gives SCCMS its name. But even at 16 months, the current was also very weak, like a much more advanced case. And the structure of his muscle cells at the nerve-muscle junction was inconsistent with the weak current. "What was unusual," says PSC senior scientist Joel Stiles, "is that he presented early in life with currents that were long and small, but the synaptic structure wasn't terribly abnormal."

SCCMS is a catch phrase for related genetic defects that lead to mutations in a protein, AChR, which plays a key role in muscle movement. A receptor protein in muscle cells, AChR works like a combination watchman and gate. It receives chemical signals from the nerve cells that tell it to open and flips the switch to become a channel for ions — mainly sodium — to flow into the muscle cell, a bioelectrical current that triggers movement. Lab studies showed a mutation in John's AChR different from any other documented case. Could this novel mutation lead to unprecedented AChR behavior that might explain John's unusual condition?

A medical doctor with a Ph.D. in physiology, Stiles specializes in computational neuroscience. With Thomas Bartol of the Salk Institute, he authored software called MCell that's used in two dozen labs around the world to simulate the microphysiology of nerve cells interacting with other cells. John's situation led Stiles to use MCell to test his hunch that the receptor was doing something other than just closing slowly.

From a series of simulations, he deduced that the AChR receptors were also slow to open. "The modeling led us to say 'Now wait a minute,'" says Stiles. "There has to be something beyond the classical picture of this disease. It has to have trouble getting into the open state as well." Lab work by Stiles's collaborators confirmed this insight into a previously unknown disease mechanism — invaluable new knowledge that can help in arriving at appropriate drug therapy as well as in research to develop new and better treatments.

## SWEEPS IN PARAMETER SPACE

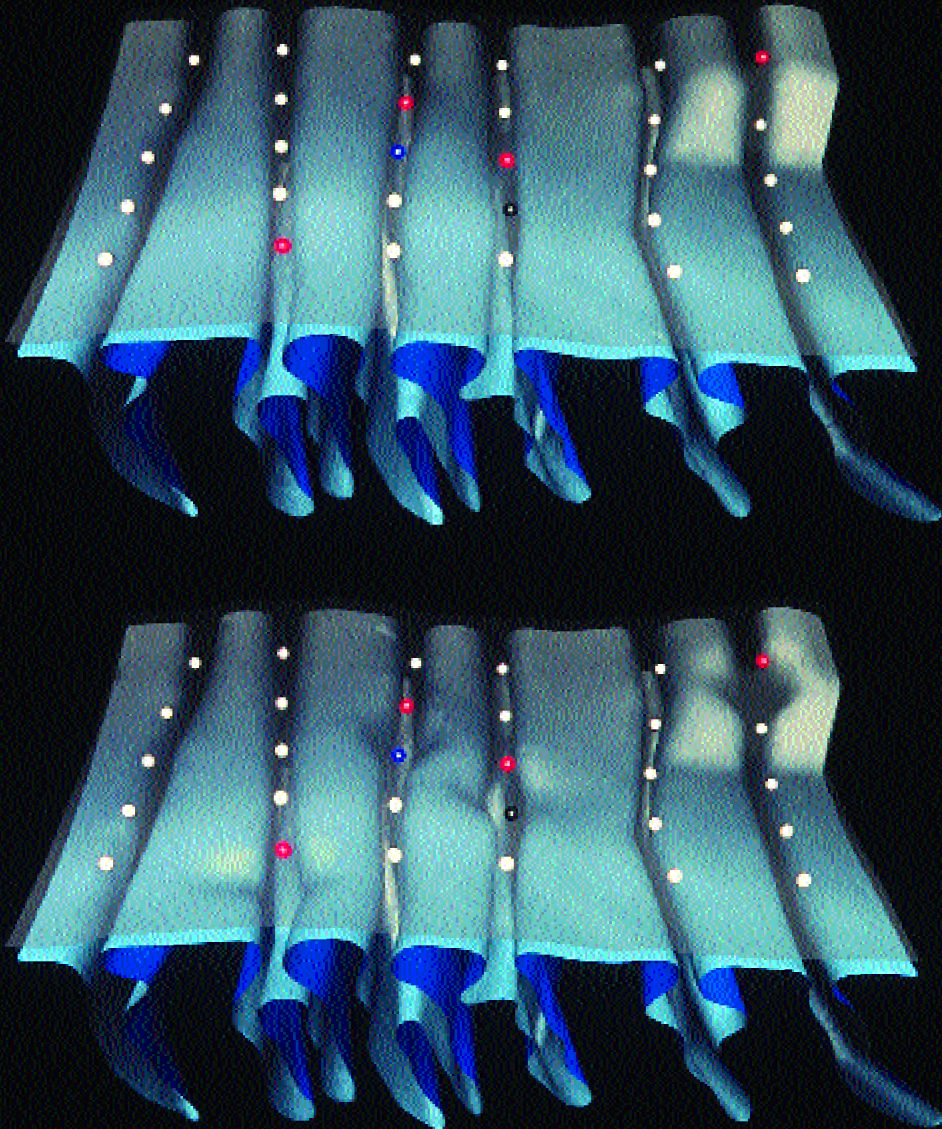
MCell is a powerful research technology that fills a gap between smaller-scale simulations that model molecules atom-by-atom and larger-scale approaches that model whole cells or groups of cells. It starts with a highly realistic 3-D model of the cellular space — reconstructed from electron microscope scans. It simulates molecular diffusion by means of algorithms that model random motion, and at each succeeding slice of time — often a microsecond or less — it uses statistical methods to test for all the possible reactions.

With an Information Technology Research grant from the National Science Foundation, Stiles and Bartol are collaborating with computer scientists at the Salk Institute, the University of California, San Diego and the University of Tennessee — under the direction of Francine Berman of UC San Diego — to expand MCell's usefulness. The idea is to create a "virtual instrument," software with an easy-to-use interface that can run on a "grid" of many different computers at the same time and keep track of thousands of simultaneous computations.

"What's embedded in this kind of work," say Stiles, "is the need to examine the parameter space the model sits in. Many unknowns go into this detailed a model — for example, the reaction between the neurotransmitter and the receptor and other proteins. If you have a model with 20, 50 or 100 input parameters, they may all need to be varied in some systematic way, and the modeler needs to examine how output varies with changes in input. This can lead to thousands of simulations going on simultaneously, what we call gigantic parameter sweep scenarios."

Stiles and Bartol and PSC systems engineer Stuart Pomerantz are working on MCell project-design issues and on a graphical user interface that will allow researchers to control these parameter-sweep simulations. At the same time, the team of computer scientists is developing sophisticated ability to "steer" them. With software that can monitor simulations in progress, the researcher will be able to shift mid-stream, with no loss of data, to refine parameters and test possibilities suggested by interim results.

**More information:** <http://www.psc.edu/science/stiles.html>



### MODEL NEUROMUSCULAR JUNCTION

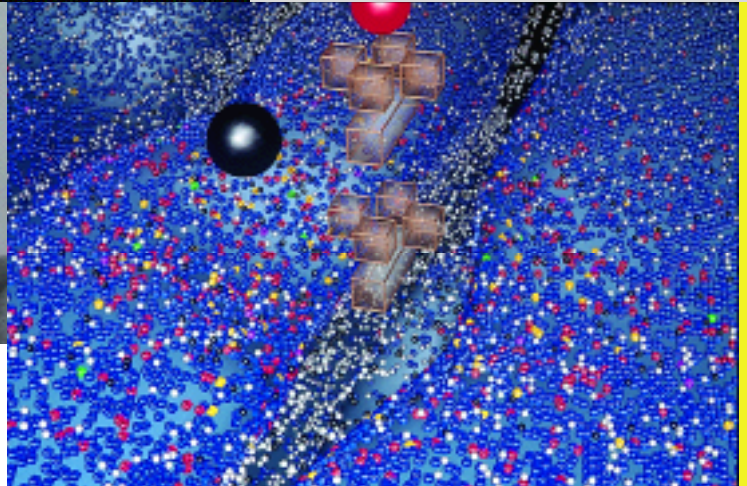
Normal architecture (TOP) contrasts with the focally deformed structure (BELOW). The localized depressions of the deformed structure represent the microphysiology of the novel SCCMS. In both cases, the model includes a nerve membrane (semi-transparent sheet) and 30 synaptic vesicles, five arrayed above each cleft in the muscle membrane. Four of these (red) are release sites for neurotransmitter molecules. Two other release sites (blue and black) are adjacent to focal deformations.

### AN MCELL CLOSEUP

Neurotransmitter molecules are releasing from a vesicle (red) into a globally deformed neuromuscular junction, representing severe, late SCCMS. Another vesicle (black) isn't releasing. The simulation tests for neurotransmitters at two sampling regions (shown as boxes). The colored markers indicate AChR receptors, in five different states of interaction with the neurotransmitters, ranging from unbound (blue), to intermediately bound (red & purple), fully bound (green) and activated (yellow). Other markers (white and black) represent an enzyme, AChE. "In a normal muscle membrane," says Stiles, "yellow would predominate because a large fraction of the receptors would be open and making current."



Joel Stiles, senior scientist,  
Pittsburgh Supercomputing Center.



MODELING THE NERVE-MUSCLE JUNCTION IDENTIFIES A PREVIOUSLY UNKNOWN DISEASE PROCESS.