SIMULATIONS OF NEURAL SYNAPSES OVERTURN SOME LONGSTANDING ASSUMPTIONS

FIRE IN THE BRAIN
Each time Sergei Rachmaninoff sat down to perform his virtuosic Piano Concerto No. 3, he played more than 28,000 notes in about 30 minutes, his fingers, a blur of muscular energy, striking the keys with the precise force — at the precise instant — the maestro intended. With only faint electrochemical signals running through nerves of greatly varied length, how is it possible for the brain to initiate and control such rapid-fire movement, dozens of muscles simultaneously, with exquisite sensitivity and split-second timing?

Over the past century, scientists have taken many steps toward understanding the biology underlying such feats. The synapse — where nerve cells meet or connect to a muscle fiber — amplifies the tiny voltage generated in the brain into forces large enough to pound out a thunderous finale. Still, these intricately coordinated mind-body processes hold many mysteries.

PSC senior scientist Joel Stiles and his collaborators solved a few recently by using a supercomputing system to recreate the business end of a synapse. Their tools included MCell, powerful software — co-authored by Stiles and Thomas Bartol of the Salk Institute — for simulating the microphysiology of interacting cells, coupled with DReAMM visualization software developed in Stiles’ lab at PSC. The computational work relied heavily on Jonas, PSC’s 128-processor shared-memory HP system dedicated to biomedical research. Stiles and colleagues modeled two kinds of synapses in unprecedented detail, and their findings, published recently in Science and other journals, overturn some longstanding assumptions about neural communication, offer insight into a family of crippling diseases, and demonstrate the power of computation allied with experimental measurement.

A Barrage of Neurotransmitters

The secret to coordination, says Stiles, is predictability. As a child grows, the motor cortex in the brain develops increasingly sophisticated programs of voluntary muscle control. These programs must anticipate delays that occur as a command impulse travels to the spinal cord, out a peripheral nerve, and across the nanometers-wide synaptic cleft that separates a neuron from the muscle fiber it innervates.

Decades ago, biologists measured the signal delays introduced by nerve-muscle synapses, and found that the lag time is amazingly consistent, varying less than 30 millionths of a second from one synaptic firing to another. How does the body manage this feat of consistency?

Over the years, molecular biologists have filled-in much of the story. On its way from brain to limb, an impulse shoots down the nerve cell to its terminus and arrives in the form of a rapid change in sodium ion concentration. That creates a voltage. The voltage triggers thousands of sphincter-like proteins embedded in the cell wall of the neuron; these protein channels open, and some allow calcium ions to flow into the cell. As the calcium ions enter, they diffuse throughout the interior of the neuron, bumping into tiny spherical containers — called vesicles — arranged in neat rows close to the interior wall.

Loaded with the neurotransmitter acetylcholine, the vesicles are like a battery of fireworks poised to launch a neurotransmitter barrage into the synaptic cleft — where this amplified signal will in turn energize the adjacent muscle fiber. In the last step, what biologists call a “fusion event,” the calcium ions trigger some of the vesicles to fuse with the cell membrane and open outward, allowing their payload to escape.

The details of this critical last step, however, have so far stumped neuroscientists. “We still don’t know how calcium binds to receptor areas on the vesicles to lead to this fusion event,” says Stiles. “Nor do we know how many protein channels need to open to admit the calcium, or how far the calcium can diffuse to find the vesicles and cause them to fuse. Does a calcium channel have to open right next to a vesicle, or can the ions come in through a variety of different gates and collectively trigger a variety of vesicles?”

Two Active Zone Architectures

Cutaway views into the interior of a nerve cell show low (top) and high magnification views of two different active-zone configurations simulated with MCell by Stiles and colleagues. The simulations include diffusing calcium ions (small blue dots) and synaptic vesicles (large purplish spheres) with calcium binding sites (red & yellow) and calcium channels (color-coded glyphs in blue triangles) in the nerve membrane. This model has a small number of binding sites on each vesicle. Translucent boxes are used to count calcium ions in different regions of space. (DreAmm image by Joel Stiles, PSC).
Neurotransmission in the Ciliary Ganglion

A nerve from the brain leads to the ciliary ganglion, which controls the iris and lens of the eye. This visualization from an MCell simulation represents neurotransmitter molecules (small green ovoids) diffusing from vesicles (translucent yellow spheres) in a reconstructed ciliary ganglion synapse. Different neurotransmitter receptors (small red circles & blue squares) are also represented. This simulation showed that neurotransmitters release at ectopic (non-active zone) sites as well as from active zones. (DReAMM image by Thomas Bartol, Salk Institute).
A New Picture of Fusion

To help answer these questions, Stiles teamed up with PSC researcher John M. Pattillo (now at Macon State College) and neurobiologist Stephen D. Meriney of the University of Pittsburgh. With support from the National Institutes of Health, the group was able to take a hybrid approach that combined classic empirical observation with a sophisticated Monte Carlo supercomputer simulation.

Stiles and Pattillo used computer-aided design tools to create a three-dimensional model of an entire “active zone,” the region inside the neuron where dense arrays of vesicles dock and fuse. Then, using MCell they created a simulation that tracks each calcium channel, the calcium binding sites on vesicles, and thousands of diffusing calcium ions inside the micron-wide active zone for several milliseconds of the firing cycle.

In the laboratory, Meriney probed living nerve and muscle cells to record how calcium concentration spikes and then falls following an electrical impulse. “These and other experimental results constrain many of the variables,” explains Stiles, “leaving us with only a few free parameters to play with in the simulations.” Running the simulations over and over, Stiles and Pattillo looked for combinations that would reproduce the recorded behaviors of real synapses: for example, the short and consistent delay between the stimulating impulse and neurotransmitter release.

Long used in high-energy physics, astronomy and other areas of science, such a hybrid approach to modeling, notes Stiles, has been less used in biology. “In part, this is because biology is so complicated and difficult to measure on these scales, and in part because the computational cost is so high. We are only now getting to the point where we have the supercomputer power and the insight into biomolecular dynamics to do computational biology this way.”

To thoroughly explore the plausible range of permutations, Stiles and Pattillo had to run roughly 500,000 simulations. Each run generated thousands of output files, so the group devised a compression scheme, analogous to the MPEG encoding used for DVD movies, that allowed them to efficiently store the results and mine them for insights.

After more than a year of patient work, the data mining struck gold — in a surprising place. Neuroscientists had for years guessed that each synaptic vesicle sports four binding sites for calcium ions, and that fusion occurs only when ions dock at all four. This was one of the first models Stiles and Pattillo tried. “The results made it immediately obvious,” recalls Stiles, “that this wasn’t right.” The virtual neuron almost never released transmitter, and no amount of tweaking other variables could produce realistic behavior. “We scratched our head and said, ’OK, let’s push up the number of binding sites on each vesicle and see what happens.’”

After testing many different combinations, the group finally discovered one that neatly reproduces the experimental data. In this model, each vesicle is dotted with 25 to 40 binding sites, and fusion occurs when calcium ions fill six to eight of those sites. “The latest data coming in from biochemists now suggest that there are good reasons to expect this is true,” says Stiles. “So that is quite gratifying.”

The achievement builds on another project to which Stiles also contributed, in collaboration with Terrence J. Sejnowski, Thomas M. Bartol and others at the Salk Institute and the University of California, San Diego. That effort similarly constructed a 3-D model of a synapse, in this case one that connects two neurons. Using a model derived from microscope cross-sections of actual synapses, the simulations overturned the conventional view that vesicles release neurotransmitters only within the active zone. Fusion events, they concluded, must be occurring in other regions of the synapse as well.

Such detailed insights into the structure of synapses are especially relevant for a class of diseases, called myasthenias, that arise when synapses are malformed or attacked by the immune system, leading to weakness, motor dysfunction, even paralysis. Because there’s a need, and because MCell has proven abilities, prospects are promising for this way of understanding cell-to-cell interactions. With a soon-to-be-released new version of MCell and DReAMM, even more precise answers will be possible. “In the new version, molecules are able to react chemically with each other,” says Stiles, “as they diffuse through space. So much more general phenomena now become potential subjects for MCell simulations.”

MCCELL SIMULATIONS HAVE PROVEN ABILITY AT REVEALING THE DETAILS OF CELL-TO-CELL INTERACTIONS