Neuron

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Obituary

Lawrence Baruch Cohen (1939–2023)

There was a generation of neuroscientists, having started their career working with "squishies," that made Woods Hole their home each summer. Lawrence Baruch "Larry" Cohen, who passed in May this year at the age of 83, was at the heart of that generation. He maintained much of his laboratory at Yale—encompassing optical tables and a menagerie of electronic equipment bolted into nineteen-inch racks—on wheels so that he could readily make the pilgrimage to and from Woods Hole with a minimum of set-up time. And he did this for the better part of 50 years. Every summer weekday at noon, on all but the rainiest days, Larry could be found holding court by the wooden benches outside of the Marine Biology Laboratory's Rowe Laboratory (né Whitman). Spreading out his sandwich and joined by colleagues, Larry would relish discussing the latest gossip—science and not —and especially appreciate sharing a good joke. It was there, as a newly minted PhD and student in the Neurobiology Summer School in 1984, that I first met Larry. My teacher, the late Ed Furshpan, had told me "*There's a physiologist named Larry Cohen who likes to work with physicists, so maybe he'll talk to you!*" and "*You'll recognize him, he always wears a red shirt.*" Both statements were true. And so we befriended each other, and a decade-plus later, I had the pleasure of joining Larry's long list of summer-time collaborators as we imaged electrical waves in a vertebrate brain.[1] Beyond the shared joy of new science, Larry pushed me to think about experiments in terms of a ''standard experimental approach'' that was then coming of age in neuroscience: identify a behavior of interest; measure the underlying neuronal dynamics, and, in something more readily accomplished with today's neuroanatomical and connectomic tools, trace the underlying circuit.

Larry brought optical imaging of neuronal activity to neuroscience. His odyssey began as a postdoctoral fellow, together with Bertil Hille, in the laboratory of Richard Keynes at the University of Cambridge. In 1968, Bertil and Larry reported on changes in the optical birefringence and light scattering of axons during and after an action potential. These effects are of interest to understand how the action potential transiently reorders lipids in the membrane and shifts the water balance in the axon. Yet their magnitude is small, and at least so far, activity-dependent changes in the intrinsic optical properties of neurons are of unclear practical use for the study of neuronal networks. Larry realized that changes in membrane potential could best be probed through optically active molecules that spanned the membrane and served as indicators of voltage. The guiding notion is the Stark effect, where a change in the transmembrane field can shift the optical absorption of a dye. In fact, also reporting in 1968, Ichiji Tasaki and collaborators discovered changes in fluorescence from axons that were stained with 8-anilinonaphthalene-1-sulfonic acid, used to study protein folding, whose rise time coincided with the onset of an action potential. Unfortunately, the signal-to-noise ratio was $\sim$0.01 per spike, far from that needed to observe single events. Nonetheless, motivated by this early work, Larry set his eyes on molecules intrinsic to the membrane, on a plethora of laser, fabric, and food dyes available from Eastman Kodak and other companies, and on a few amphipathic dyes readily synthesized. The venerable squid axon provided a testbed. More than one thousand different dyes were tested as potential molecular indicators of membrane voltage. In retrospect, a significant act of faith was involved. The sought-after dye was expected to label the outer membrane but avoid labeling organelles, which would contribute only to the background signal and associated noise. The dye, whose interaction with the membrane potential depends on an internal dipole moment, could not significantly alter normal electrophysiology. Lastly, the change in optical properties was ideally detected through a change in fluorescence rather than absorption, both so that the signal is measured with respect to darkness rather than background light and so that the dye can be used to label optically thick tissue. All in all, a tall order.

Lawrence Baruch Cohen. Photo credit: Jennie Cohen.

Larry's perseverance was rewarded. His intermediate results were reported as a series of meeting abstracts in the *Biological Bulletin* in 1970 and 1971. The following year, he and his then postdoctoral fellows Vicencio Davila and Brian Salzberg hit paydirt. Their work on the optical recording of voltage changes during an action potential in the squid axon, with a large enough signal-to-noise ratio to be observed in real time, was published in 1973. It was followed in the same year by the optical recording of action potentials from individual neurons in the ganglion of a leech, a "simpler" nervous system.[2] This marked a watershed event—the use of fluorescent microscopy, with an array of solid-state photodetectors, to measure from afar what once was possible only with mechanical manipulators and invasive glass electrodes.

Brian recalls the halcyon days of testing dyes and, in 1972, their first significant discovery.

> We had assayed more than one hundred fifty colored molecules for voltage sensitivity, using the voltage-clamped squid giant axon,

nearly exhausting the fluorescent molecules listed in the *Dyes and Colourists Index*, with the dye Nile Blue A being our best candidate. At that point Gary Strichartz suggested that we might examine merocyanine dyes; Gary had worked on these dyes as a graduate student with Britton Chance. Larry and I wrote to Eastman Kodak, who had synthesized many merocyanines as photosensitizers, and they responded by sending us four dyes and their structures. Vicencio and I chose one of them as our first candidate and tested it for voltage sensitivity on the squid giant axon under voltage clamp. The five seconds that it required to reduce the oscilloscope gain by more than an order of magnitude to accommodate the gigantic optical signals were the most exciting five seconds of my scientific career. Yet we continued to push, and screening for ever better dyes was augmented when Bill Ross joined Larry's laboratory as a fellow in 1974.

The existing dyes were sufficient to start this approach, but the signal-to-noise ratios were still limited in the initial studies. The late Amiram Grinvald, a physical chemist by training and an early postdoctoral fellow with Larry, was quick to address this limitation. From his then new position at the Weizmann Institute, Amiram formed a long-term collaboration with organic chemist Rina Hildesheim to develop new molecular indicators with ever increasing sensitivity to changes in membrane potential. Rina notes, ''Larry's spirit was in the laboratory as we conceived, synthetized, and tested new molecules.'' The new dyes from Rina, and later dyes developed by Kohtaro Kamino, Leslie Lowe, and Alan Waggoner, led to relatively high signal-to-noise ratio measurements of transmembrane voltages. By the mid 1980's, two milestones occurred in the growing impact of voltage sensitive dyes on new science.

Working both separately and in collaboration, Larry and Amiram used Rina's purposely synthesized voltage-sensitive styryl dye, RH-414, to record population activity in vertebrates. These works reported a new kind of measurement. As best could be determined, the organic voltage sensitive dyes permeated the entirety of the membrane of all cells—neurons, glia, microglia, endothelia cells, etc.—albeit with different affinities. At the level of neurons, signals originate from the relatively slow changes in the membrane potential dendrites, the fast as well as slow changes in the membrane potential somata and the fast transient changes in axons. Thus, each spatial patch of fluorescence from voltage sensitive dyes contains a composite signal that is different from anything recorded with an electrode, i.e., a mix of fast and slow contributions from voltage changes in individual neurons along with contributions from action potentials in axons of passage. Despite the seeming complexity of the new signal, these novel measurements enabled the observation and characterization of large-scale activity in brain slice, tectum (superior colliculus), olfactory bulb, and neocortex, with publications as early as 1983 and continuing for another two decades. This included the expansive nature of depolarization across neocortex induced by a punctate stimulus, such as the flash of a point of light or the flick of a single vibrissa.[3]

A particular milestone set by Larry concerned the decoding of neuronal activity during a behavior on a cell-by-cell basis. These are prescient studies on the population dynamics of many synaptically coupled neurons. Larry and his colleagues, notably Jian-Young Wu and Dejan Zecevic, made use of invertebrates with their stereotypic natural behaviors. The underlying motor actions are controlled by tens to hundreds of neurons arrayed as a relatively thin sheet across specific ganglia. This geometry allowed Larry to isolate the signals from many, albeit not all, of the individual neurons that contributed to motor actions. For the case of the marine gastropod mollusk Navanax, the animal captures other mollusks with a pharynx that protracts and seals to the prey and then expands to engulf it. Larry showed that the neuronal drive for this sequence is represented by tens of neurons in the buccal ganglion.[4] It is of note that the neuronal responses are distributed across many neurons, consistent with then existing ideas about ''switchboard'' networks in nervous systems, but not previously observed. Further, the temporal patterns of neuronal spiking across different neurons appear as a sequence of states, i.e., recognizable intervals in which different but overlapping sets of neurons spike (Figure 8 in London et al.[4]). While John Hopfield had published his now famous manuscript on attractor neural networks in 1982, the notion of a neuronal ''state'' was far outside the mainstream of the neuroscience community at the time of Larry's experiments. Larry went on to measure the distributed nature of spiking for other behaviors, including gill withdrawal in the marine gastropod mollusk Aplysia. Time would pass until the computational tools to readily identify potential attractor states came of age. Yet one can argue that Larry's work catalyzed experimental studies on behavior in terms of finite state controllers and other contemporary ideas.

Larry understood the need to bring voltage-sensitive dyes into the realm of genetic expression. Ehud Isacoff had published on a genetically expressible voltage indicator back in 1997, constructed by linking a cyclically permuted GFP to a subunit of the A-type potassium channel that physically translated between hyperpolarized and depolarization voltages. While a pioneering step, the indicator exhibited a relatively slow response and did not express in mammalian cells. Larry, taking a different approach, teamed with Vincent Pieribone and produced a sensor that linked the Ciona intestinalis voltage sensor, which apparently changes shape with voltage, to a pH-sensitive GFP. They christened this ArcLight.[5] The resulting construct expressed well in mammalian neurons; excitatory post-synaptic potentials as well, as action potentials were readily observed in cultured neurons and recorded in real time. The Allen Institute for Brain Science went to on make mouse reporter lines with advanced constructs of ArcLight. Larry, who has recently divided his efforts between Yale and the Korea Institute of Science and Technology, was in the middle of experiments on the concentration invariance of odor recognition at the time of his passing.

Larry remained a thoroughly modest and unassuming man throughout his life. He also lived as much as an ''academic socialist'' as one could and still be a working scientist. Experiments were often

performed as a group, following a laboratory-wide discussion among Larry and his proteges. For many years, he and his family lived in a group house in North Haven with three other families. Dinner parties at Larry and Dejan's co-owned home in Woods Hole brought together scientists from all over the world. My family and I certainly remember Larry beaming while serving his whole roasted fish, having been purchased fresh from the local market. Lasting friendships were formed over these dinners.

It has been 50 years after the first fluorescent measurement of spikes produced by a neuron. Genetically expressed indicators of voltage, along with neurotransmitters, neuromodulators, and second messengers, especially Ca^{2+}, are now viable and continually improved tools. Bulk labeling has given way to the expression of indicators by specific cell phenotypes and/or cells with specific patterns of axonal projections. Optical sectioning with one-photon light-sheet microscopy and two- and three-photon laser scanning microscopy has largely supplanted widefield fluorescent imaging to observe populations of neurons. And techniques from machine learning have largely replaced graduate students and postdoctoral fellows checking off individual spikes across reams of data. Exciting times. Much of it started by the man in a red shirt.

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