ESSAY

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Influences: Short circuits

David E. Clapham

I grew up a couple of miles above a reservoir of hydrocarbons called the Permian Basin, the surface of which is known as West Texas (think *No Country for Old Men*). After moving to Bolivia and then Colorado, I ended up as an electrical engineering student at the Georgia Institute of Technology in the hope of becoming a computer whiz. Punch cards and batch processing killed my interest in programming but led me to think about other pursuits. I latched onto the idea of being a doctor in Africa: idealistic, but sincere. During my senior year, while trying to acquire enough biology-oriented credits for medical school, I entered the classroom of a most peculiar character.

Lou DeFelice was a physicist turned biophysicist who taught at the Emory School of Medicine and Georgia Tech. His class was unique to me; he tried to engage the students by thinking about the unknown rather than lecturing about the known. He enlightened me to the fact that cells used electricity in interesting ways. From the day that I visited his laboratory and saw cardiac action potentials in real time, I was hooked on physiology.

Before I could begin real-world experiments, however, I had promised Lou that I would linearize the Hodgkin-Huxley, Hille, and Beeler-Reuter sets of nonlinear differential equations (à la Knox Chandler and Richard Fitzhugh [1]). These calculations were needed to derive the transmembrane impedance for small signals across the membranes of squid axons, nodes of Ranvier, and heart cells, respectively. These numbers, as functions of voltage and frequency, were used to estimate the channel conductances underlying some of Lou's current noise recordings. At that time, the only way to directly measure single ion channel conductance was to either put purified channel proteins into lipid bilayers or make measurements of membrane current noise under voltage clamp. Lou and I shared a fear and loathing of biochemistry and bilayers (practiced by scurrilous characters such as Chris Miller [2]), which left real measurements as the only way ahead for our experiments.

Months passed. I got married and started medical school, and my wife, Susan, started working for Lou to help him turn his notes and calculations into a book called *Introduction to Membrane Noise* (3). The resulting 500-page monograph could hardly be called an introduction, but it kept me interested in reading and calculating, and it kept Susan (who was, after all, footing the bill for our lives) employed. Ever in need of funds (little did I know at the time that this was to become an intrinsic part of being a scientist), I became an MD/PhD student, working with Lou DeFelice and Bob DeHaan on cardiac electrophysiology.

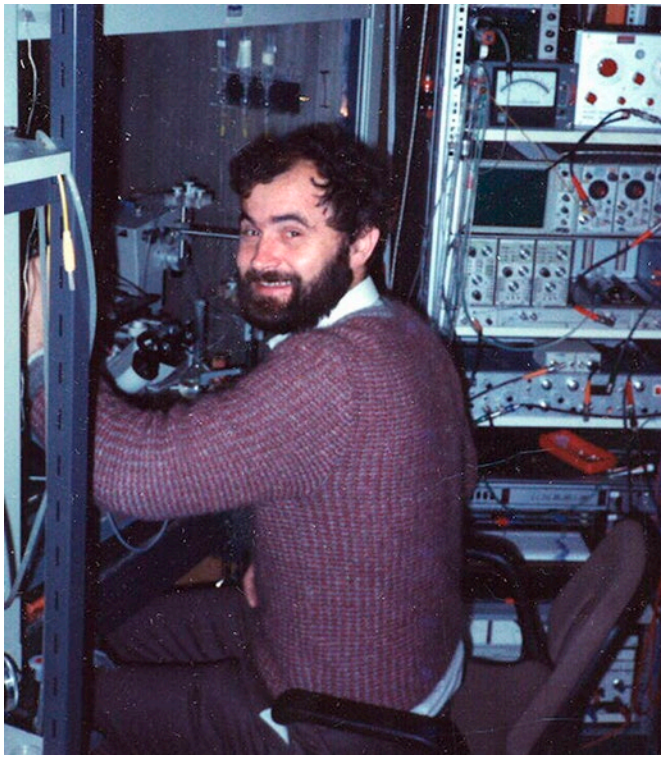
Lou DeFelice in enthusiastic mode. Photo courtesy of the author.

The years working with Lou molded and changed me in all sorts of ways. First, we became good friends; Susan and I babysat the DeFelice kids in exchange for use of their washer/dryer on Friday evenings. Before and after their respite from children, we enjoyed socializing with Lou and Rachel; our conversations almost always ended up being about science, in a "Mr. Wizard"[1] sort of way. For example, during one dinner conversation, Lou got excited about seeing whether a powerful magnet (handily available) would alter the image on his television screen. The magnet, however, permanently altered the phosphorescent screen of the television's cathode tube, a lasting reminder of the incident, to

Howard Hughes Medical Institute, Chevy Chase, MD.

Correspondence to David E. Clapham: claphamd@janelia.hhmi.org.

[1]American television program (*Watch Mr. Wizard*) of the 1950s demonstrating the science behind ordinary things.



Erwin Neher enjoying experiments. Photo courtesy of the author.

the annoyance of the DeFelice children. Lou was always full of crazy ideas based on physical principles, such as making an ideal vibration isolation table by suspending a marble slab from a single spring, tuned to a frequency we could then electronically filter. We spent a weekend constructing the contraption and came to understand that there were unexpected difficulties, such as having to hover above and move with the gently swinging table while placing a sharp electrode into a cell the size of a few microns.

Before patch-clamp recordings, voltage clamp (pioneered by K.C. Cole) was the technique to measure current across cell membranes, but for mammalian cells, it required the insertion of two sharp electrodes into the cell. Hours or days were needed to obtain a single good recording. The resulting currents were large, and single-channel conductances could only be estimated by the noise analysis that Lou had helped to pioneer. In 1976, Lou and I discovered a paper by Erwin Neher and Bert Sakmann (4) that reported single nicotinic channel currents from frog muscle. We began to look for single channels ourselves on cells. But just like in Neher and Sakmann's first experiments, we had trouble getting good enough contact through high resistance seals between cell and electrode; these seals were needed to reduce the noise to picoampere levels. The problem was our choice of red blood cells from which to record; despite many technical advances, these cells are still notoriously difficult to patch clamp. In typical contrarian fashion, we resorted to centrifuging single cells down into the tip of glass electrodes, where they would jam in the tip. We never did see single channels. We did occasionally "clog" the electrode, making probably some of the first (but unrecognized) gigaohm seals on channel-less patches of membrane. I remember Lou remarking on the "little bees" around the red cells, which, as a learned medical student, I pointed out were bacteria.

After finishing up the research portion of my MD/PhD program, I spent a few weeks with Lou at Woods Hole to record current noise across squid axon membranes in Bill Adelman's laboratory. I learned to voltage clamp these axons, but also had a mild spat with David Goldman over the use of time on the Woods Hole computer (yes, there was only one). We also enjoyed interesting conversations with Alex Mauro and other visitors that regularly passed through that special place. I loved the Woods Hole atmosphere of total dedication to experiments and think fondly of my brief time there.

With my medical school training nearing its completion, I spent a month-long externship at Yale to learn to read electrocardiograms but also explore a potential residency and postdoctoral fellowship with Dick Tsien or Chuck Stevens in the Physiology Department (5). Being socially dyslexic, I think I managed to offend both of these outstanding researchers by my stated enthusiasm for working with Erwin Neher. Sadly, they were deprived of my skills and had to resort to Bruce Bean, Rick Aldrich, Gary Yellen, Peter Hess, Rocky Kass, Steve Siegelbaum, Dick Horn, and other "reasonably good" postdoctoral fellows for help.

The 20 or so medical student externs visiting Yale were all housed on one unisex floor in Harkness dormitory. Most went to local restaurants for dinner, but having no money, I stayed in the dorm and ate peanut butter sandwiches. On the bright side, I was the only one on the floor to avoid being mugged, which was frequent in New Haven in the late 1970s. During that externship, I did manage to make a little money on a state-variable filter electronic stethoscope I designed, which funded the next adventure: a brief trip to Göttingen, Germany, to explore a postdoctoral fellowship with Erwin Neher.

In the 1970s, biology laboratories had a principal investigator and a couple of postdoctoral fellows or students. This model was possible because of the simplicity of techniques used at the time and enforced a close working relationship that benefitted everyone. When I visited the Max Planck Institute in Göttingen, Erwin's laboratory had two postdocs and a technician. One postdoc, Alain Marty, was about to leave for a faculty position; the other, Fred Sigworth, was busy working on the design of patch-clamp amplifiers and recording ensemble-averaged sodium channels. Alain and Erwin sat with me for a couple of hours to show me how to obtain giga-seal recordings, and Erwin agreed to take me for a postdoctoral fellowship. Alain lent me 20 Deutsche Marks for dinner, which I still owe him, and I journeyed back to life as a resident in internal medicine in Boston.

I began my residency at Brigham and Women's Hospital (BWH) in Boston in 1981. BWH was partial to MD/PhD residents; Marshall Wolf, the legendary director of the residency program, allowed me to take some time after my internship year for a postdoctoral fellowship with Erwin Neher in Göttingen. Like Lou, Erwin was a physicist turned biologist and a remarkable person. He was kind, encouraging, patient, and incredibly smart. During a most exciting time in physiology, every day working with Erwin was a privilege. A side benefit of being in Göttingen was meeting scientists interested in the new method of patch clamp (6), such as Bernard Katz. Later in my 1.5-year fellowship, we were joined by Julio Fernandez and Walther Stühmer. We shared lunch daily with Bert Sakmann and his laboratory members: Owen Hamill,

Yoshi Kurachi, and Joachim Bormann. These researchers all became lifelong friends, and on occasion, we later performed experiments together.

35 years later, I was adapting to my new job at the Howard Hughes Medical Institute in Washington, DC, when I got a phone call from Scott Ramsey, a former postdoc in both my laboratory and Lou's. Scott told me that my lifelong mentor, Lou DeFelice, had just died unexpectedly while working in the laboratory. The swinging vibration table, the destroyed television, and dozens of other images went through my mind. It struck me that I had never thanked or in any way repaid Lou for all the advice and scientific fun that we had shared. Lou and I had almost weekly conversations over those 35 years, usually focusing on crazy ideas, often trying to understand new and old physics. No matter how difficult my life had seemed at times, Lou's scientific imaginings were always a happy respite. Then I remembered those first cardiac action potentials; they don't go on forever.

Here is what I learned from having the great privilege of working throughout my scientific lifetime with Lou DeFelice: life is short, friends are precious, science is sacred. Constantly explore new ideas, work with honest people, and forgive their failings. Be generous and tolerant.

Acknowledgments

I thank Chris Miller for being impervious to insult.

Lesley C. Anson served as editor.

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