ESSAY

Influences: The Cell Physiology Laboratory in Montemar, Chile

Francisco Bezanilla[1,2]

Looking back, I was really lucky. In 1967, Dr. Joaquin Luco arranged for me, a student from the Catholic University, to join the Laboratorio de Fisiologia Celular in Montemar to work on my doctoral thesis under the supervision of Dr. Eduardo (Guayo) Rojas from the University of Chile. I had completed three years at medical school and three years at engineering school, but my laboratory experience basically comprised short encounters with research in Joaquin Luco's neurophysiology laboratory and with the squid giant axon (Fig. 1) in Francisco (Pancho) Huneeus' laboratory across the street. But the Montemar laboratory was very different. The approach was quantitative and dealt with a subject that had fascinated me since I was in high school, when I learned for the first time that the nerve impulse was an electrical event. Electricity had been my hobby since I was in junior high, learning by doing as I moved from crystal radios to transmitters and TV sets. I became a radio amateur, not so much to contact other hams, but as an excuse to build a more powerful transmitter that could reach farther away, learning electronics in the process. At that time, everything I built was with vacuum tubes. When I arrived in Montemar, the first assignment from Guayo was to build my own setup with parts available in the laboratory, including micromanipulators, a microscope, pieces of plexiglass, and (wow!) solid-state operational amplifiers. For me, this was like arriving in paradise. I immediately built the voltage clamp with Philbrick op-amps and vacuum tubes in voltage follower configuration as amplifiers for the internal and external electrodes, which I replaced later with operational amplifiers.

The laboratory is located in Montemar, on the coast of Chile, a few kilometers north of the city of Valparaiso. The laboratory, which had previously been a brothel and conveniently had plumbing in all rooms, was bought by the rector of the University of Chile at the request of Dr. Mario Luxoro to establish a research laboratory for scientists working on the giant axon of the Humboldt squid *Dosidicus gigas*. This large squid, which can reach almost 2 m in length, was caught offshore by fishermen using small boats launched from the marine station ~100 m from the laboratory. Guayo and Mario had previously worked at this station, where they showed that a protease injected into the axon abolished action potentials but not the resting potential, demonstrating for the first time that proteins played a fundamental role in ionic conduction underlying the nerve impulse (1). However, the rules of the station's director at that time were incompatible with the schedule of experiments and squid collection, which prompted Mario to procure the new laboratory from the rector of the University.

Figure 1. **Opening the mantle of a Humboldt squid in the boat.** Insets show a dissected axon (scale in cm, top) and an axon being mounted in the recording chamber (bottom).

The Laboratorio de Fisiologia Celular had two floors on which rooms had been converted into laboratories for Mario, Guayo, Dr. Mitzi Canessa, Dr. Siegmund Fischer, and Dr. Fernando Vargas. All these principal investigators used the giant axon from the Humboldt squid as their biological preparation because the axons are so large (typically 1 mm in diameter and 20 cm long), and there are several giant fibers on each side of the squid. The projects, involving electrophysiology and biochemistry, varied from the sodium-potassium pump to streaming potentials to ion

[1]Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL; [2]Centro Interdisciplinario de Neurociencia de Valparaíso, Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile.

Correspondence to Francisco Bezanilla: fbezanilla@chicago.edu.

Figure 2. **The three graduate students of Guayo Rojas.** Guayo (center), Ramon (left), Cecilia, and Pancho (right).

Figure 3. **In the laboratory.** Illani Atwater (foreground) and Bob Taylor.

conduction. The laboratory was busy day and night because the experiments depended on squid collections, which were done at night. The fishermen, hired by the University, traveled about one hour from shore to catch the squid. But because only axons that remained on the mantle for less than one hour after capture were viable, the fishermen immediately separated the mantle into coolers containing sea water and sea water ice, and returned quickly to expecting researchers who promptly extracted the axons to be stored in cool sea water.

At that time, Guayo had three graduate students in Montemar: Ramon Latorre, Cecilia Hidalgo, and me (Fig. 2). The three of us had continuous interaction in the friendly environment of the laboratory. Indeed, Ramon and I became very close friends while we were there, a friendship that has endured the last 50 years with many collaborations on several projects. Ramon and Cecilia were working on the effects of temperature on the resting potential and the transport of nonelectrolytes in a small room on the first floor that had no windows; they published their results in *Nature* and the *Journal of Physiology* (2–4). Later on, Ramon and Cecilia's laboratory became the machine shop of the laboratory. My initial project was to measure the transference number of several ionic currents, a project that required recording currents under voltage clamp while measuring the isotopic flux at the same time in the axon. We had scintillation and gas flow counters in the laboratory that were shared by all the investigators; I used Cl^{36} and found out that the transference number was only 0.05, i.e., there is very little chloride conductance in the axon. I then started to measure the transference number of the Na^+ currents under voltage clamp using Na^{22}, which became my thesis project (5, 6).

Guayo was a superb teacher. Because he was an excellent experimentalist, he taught me by doing everything himself: how to handle the axons, perfuse them, and collect data. But most importantly, I learned from him how to analyze data and apply all the theory I had learned into experimental results. In other words, he taught me how to start thinking in the language of electrophysiology. I also learned about the experimental preparation from Dr. Illani Atwater, who worked with Guayo. We worked in a room with two setups that had a large window with a direct view of the ocean and, as the analysis was done by projecting film with an enlarger, it was mostly done at night.

The laboratory attracted scientists from the United States and England. Dr. Robert (Bob) Taylor came every year to work in Montemar and collaborate with Guayo (Fig. 3). He was the chief of the Laboratory of Biophysics at NINDS (National Institutes of Neurological Diseases and Stroke, National Institutes of Health [NIH]) and had enjoyed a distinguished career, having worked with A.F. Huxley on the local activation of muscle fibers. I met Bob as soon as I arrived in the laboratory, and because my English was quite primitive at the beginning, it was hard for me to follow him. But he was extremely patient with me, and he taught me the meaning of I-V curves, negative conductance, space clamp, and cable theory. In fact, Bob was one of the most influential teachers in my training as a biophysicist, and his rigor and physics training shaped my scientific career. Almost a decade later, Bob and I began a collaboration, which lasted until his death, at Woods Hole during summer squid seasons and in Los Angeles in the winter. Dr. Clay Armstrong also came to Montemar every year (Fig. 4). Clay worked independently, so had his own setup in the laboratory, and it was there that he did a large fraction of his research on TEA and derivatives, which shaped our understanding of ion channel gating and permeation. Clay's approach was mechanistic and his remarkable intuition allowed him to envision and imagine structural details of ionic channels that at the time were only hypothesized (7). He frequently came to our room and had conversations with Guayo that were extremely enlightening to me. One of them was about the effect of pronase, which he correctly proposed to affect inactivation of the Na conductance (8). Clay, of

Figure 4. **Clay Armstrong.** Clay's work on TEA derivatives done in Montemar shaped our understanding of ion permeation and gating.

Figure 5. **View of the Pacific Ocean from the first floor of the laboratory.** Through the windows of the first floor, a large wave washed the interior of the laboratory in 1968.

course, was the other most influential person in my formation as a scientist. I would like at this point to repeat my initial statement that I was lucky to come to the Laboratory in Montemar at that particular time; Bob, Clay, and Guayo all played a role in shaping my future. In particular, Bob arranged my first postdoctoral appointment with himself, Dr. Richard (Dick) Fitzhugh, and Dr. K.S. (Kacy) Cole at the NIH, and Clay arranged my second postdoc with Dr. Paul Horowicz in Rochester, affording me the privilege of working with Clay in Woods Hole during the summers.

Everything was going well during 1968, until a couple of gigantic ocean waves washed the shore of the Valparaiso region and hit the Laboratory. They were so strong that the windows of the first floor were pushed inside the laboratory and all the equipment was submerged in seawater and sand (Fig. 5). A large fraction of the equipment, mostly electronics such as oscilloscopes, pulse generators, cameras, counters, etc., was salvaged by first bathing it in fresh water to remove the sand and salt and then in alcohol to dry it. Many of the items still operated after this treatment, but a few pieces of equipment were lost. Guayo, Illani, and I subsequently went to the U.S. to visit Bob at NIH, who gave us a few pieces of equipment to bring back to the laboratory in Montemar. Luckily, all this happened during the offseason of the squid, but the laboratory had to be repaired for the next season that started in November of that year. The large windows that had a beautiful view of the ocean in Guayo's and Mario's laboratories on the first floor of the house were each replaced by a wall with a tiny window in preparation for future waves.

In the following season, Guayo, Bob, and I decided to measure the time course of Na influx during the action potential, which required interrupting the action potential at different times using voltage clamp. Clay designed an electronic switch with two transistors that enabled us to switch the voltage clamp on within a few microseconds. Thus, we measured, for the first time, the time course of Na^+ and K^+ conductances during the action potential by evaluating tail currents at the K^+ or Na^+ reversal potentials, respectively, as a function of action potential duration (9, 10). By combining this circuit with simultaneous Na^{22} influx measurements, we could follow the time course of sodium influx during the action potential (11). We all had fun; squids were plentiful and I learned a lot from Bob, Guayo, and Clay. By the end of the season in 1969, I left for Bethesda to initiate my first postdoctoral training at NIH. At the Laboratory of Biophysics in Building 36, I learned to model with Kacy, Bob, and Dick before moving to my second postdoc in Rochester, NY at the end of 1969. There, I started working on excitation–contraction coupling in frog skeletal muscle in collaboration with Carlo Caputo under the direction of Paul Horowicz. And in 1970, Clay delivered the bad news that the squid had disappeared from Montemar and not a single one had been caught on the coast of Chile or Peru.

Zero squid seasons continued year after year for ∼30 yr. Of course, this changed the laboratory in Montemar. The giant barnacle (*Megabalus psittacus*) provided the new biological preparation: a giant muscle fiber that can easily reach two millimeters in diameter so that it could be voltage-clamped with the axial wire technique, similar to the axon preparation. Training of students continued in the laboratory and foreign scientists kept visiting, including Dr. Richard Keynes. Dr. Julio Vergara, who followed very similar training to mine, also did his thesis on barnacle fibers at the laboratory under Guayo's direction, before moving to the U.S. for postdocs at Duke and NIH. Because squids were not available anymore in Chile, Clay had to move to Woods Hole to continue his squid work, and he invited me to go to the Marine Biological Laboratory (MBL) to work with the giant axon. This was a completely new experience for me, and we worked together during squid seasons at the MBL for many years. I learned even more from him while we were setting up and developing the equipment that made it possible to record Na channel gating currents. In 1972, Mario invited me to join the faculty of the Department of Biology at the University of Chile. At the time, I was still in the middle of my postdoctoral training, so it was arranged that I could go there for a few months to start the position and then return to Rochester. I had the incredible opportunity of working on barnacle muscle fibers with Mario in Montemar, and

Figure 6. **The return to the laboratory in 2008.** Miguel Holmgren showing off the currents recorded from the first axon in 2008.

I continued my work on excitation–contraction coupling back in Rochester, before spending the summer with Clay working on squid axons at the MBL.

In September of 1973, there was a coup d'état in Chile, and everything changed. Science in Chile was negatively affected because many researchers left the country and the leadership of universities was taken over by the military. Nevertheless, at the beginning of 1974, I went back to Chile to continue my faculty position at the University of Chile. Julio also went back and we decided to install our laboratories in Montemar, where we were quite insulated from the complicated politics of the University but found ourselves essentially alone. We worked on frog skeletal muscle and, with parts that we both brought from the U.S., we built a new optical setup that allowed us to optically record the action potential of the tubular system of frog skeletal muscle (12). There was practically no contribution to the maintenance of the laboratory from the university and our subsistence there was possible only because Clay invited me to the MBL in Woods Hole to continue the work on giant axon gating currents during the summers. In the middle of 1976, it became clear that we could not stay much longer and we both returned to the U.S. to take permanent positions. That year was the last time I worked in Montemar for many years to come.

The laboratory of Montemar was kept open thanks to the continued efforts of Mario and Veronica Nassar, his collaborator at the time, who kept doing research there on several biological preparations while training several students, including Dr. Juan Bacigalupo and Dr. Cecilia Vergara. Guayo and Illani also helped to keep the laboratory alive for a few years, but by the late 1990,s Mario retired and went back to the Faculty of Sciences in Santiago, thus closing the Montemar laboratory.

It was at the end of 2007 when Dr. Miguel Holmgren (Fig. 6) called me from NIH to tell me that, when visiting his parents in Chile, he saw giant squids in the market. Miguel, together with Drs. Paul DeWeer, Bob Rakowski, David Gadsby, and I had been working on the fast transients of the sodium–potassium pump using the squid axon in Woods Hole. Although the description of the entry of Na^+ and its occlusion could be well studied, the potassium part of the cycle was borderline in signal-to-noise ratio. Thus, Miguel proposed to try the K^+ experiments on the pump using the Humboldt squid in Montemar. In January of 2008, we flew to Chile with the necessary electronics (much reduced in size compared with 40 years before) and contacted Mario, who opened the laboratory for us. It was depressing to see the dismal state of the laboratory where I had seen so much excitement and so many scientific discussions 40 years before. We cleaned a bench and one setup table, put together the old micromanipulators and dissecting microscopes that were still there under layers of dust, and with the help of Pancho Palma, we contacted the fisherman who brought us a squid. The first axon we tried was used to record classical Na^+ and K^+ currents. In the next one, we attempted the measurement of K^+ currents through the Na–K pump. To our satisfaction, we saw clear charge movement produced by K^+ entry into the access channel, followed by occlusion: the large size of the axon of the Humboldt squid made the difference.

Ramon, who returned to Chile in 1983, created an ion channel biophysics group in Santiago, which resulted in a revival of ion channel research in Chile (13). After a stay in the southern city of Valdivia, he moved to Valparaiso in 2008, where he helped to create and then direct the Centro Interdisciplinario de Neurociencia (CINV)—a Millennium Institute financed by the Chilean government. Ramon, who also has his heart in Montemar, together with Miguel and me, obtained an NIH grant to continue our work on the Na–K pump using the Humboldt squid in Chile. This grant, along with substantial support from CINV and the Universidad de Valparaiso, allowed the restoration of the laboratory and paid for the squid collections during the following years. We enjoyed visits from Drs. David Gadsby, Brian Salzberg, Jorge Sanchez-Rodriguez, Ana Correa, Eduardo Perozo, Qufei Li, and Andrea Brueggemann during our squid seasons, and we also had enthusiastic students and postdocs who learned the Humboldt squid axon preparation and performed experiments on the Na–K pump (14, 15).

The Montemar Laboratory has mostly been associated with the giant axon of the Humboldt squid, and its renaissance was caused by the return of these squid. Just recently, we used the giant axon as a preparation to demonstrate the synthesis of functional membrane proteins in the absence of a soma. This was only possible because of the large size of the Humboldt squid axon, which allowed the injection of foreign RNA and subsequent voltage-clamping plus the characterization of RNA species in the axoplasm by extruding it from a single axon (16). This has opened up the possibility of studying the details of protein synthesis in an axon preparation with high resolution and using a variety of techniques, with little interference from other cells.

The laboratory now has been renamed Laboratorio de Fisiologia Celular Mario Luxoro, honoring Mario, who recently died, for having been responsible for its creation and the initiation of biophysics in Chile. It is now our duty and that of new generations of young scientists to keep the flame alive and expand the heart of the place that has influenced so many scientists, including me.

I would like to thank Cecilia Hidalgo and Juan Bacigalupo for some of the old pictures. A movie that tells the story of the Montemar Laboratory produced by Cabala Productions and under the auspices of CINV may be found at: https://www.youtube.com/watch?v=KPey93HBekc.

Lesley C. Anson served as editor.

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