THE BIOLOGICAL PHYSICIST

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In this Issue

From the Editor

Here's your October issue, hot off the virtual press! Join THE BIOLOGICAL PHYSICIST as we take a tour of the Department of Physiology and Biophysics at the University of Washington. Then sit down with us for an interview with Larry Cohen, one of the pioneers in the field of voltage sensitive dyes.

Stay tuned for the December issue!

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Feature

Biophysics in the Department of Physiology and Biophysics at the University of Washington

Fred Rieke

The interface between physics and biology is a rapidly emerging research area. Experimental techniques are now available that permit the quantitative study of a wide range of biological systems – from the properties of single proteins to computations carried out by neural circuits in the brain. Many of these techniques have come directly from physics. As a consequence, we now have the opportunity to develop and test theories for how biological systems work. The theoretical side of this endeavor relies on analytical approaches, again many of which are borrowed from physics. By bringing together experiment and theory we hope to provide a set of rules governing the behavior of different biological systems.

Biophysics research in the Department of Physiology and Biophysics at the University of Washington follows this interdisciplinary tradition. Students, postdoctoral fellows and faculty come from undergraduate and graduate programs in both the physical and biological sciences, but all take a physical and quantitative approach to biological questions. We share regular interactions with members of other departments on campus, including computer science and physics. Several of the diverse research areas represented in the department are highlighted below.

Molecular Biophysics

Proteins are the basic building blocks of biological systems. Thus the physical properties of single proteins are a key factor in how biological systems work. Several labs in the department are working to elucidate the molecular mechanisms governing the behavior of ion channels – proteins that permit the flow of charged ions from one side of a cell membrane to another. These proteins are the basis of electrical signaling in cells.

The flow of current through an individual ion channel molecule can be monitored in real time to directly observe opening and closing transitions of the channel pore. Bill Zagotta's and Sharona Gordon's labs use this approach to study channels opened by cyclic-nucleotides – diffusible messengers inside a cell. By analyzing the statistics of the single channel openings and closings (Fig. 1), they obtain detailed information about the number of distinct conformational states of the channel protein, the allowed transitions between these states, and the relative energies of the various conformations. Studies of how genetic alterations alter channel behavior help refine the relationship between structure and function. This work, combined with structural information from x-ray crystallography, is rapidly advancing our understanding of how ion channels work.

Cellular biophysics

The cell membrane, cytoplasm and intracellular compartments form a rapidly interacting signaling network. These signaling networks perform diverse functions, including regulating our heart

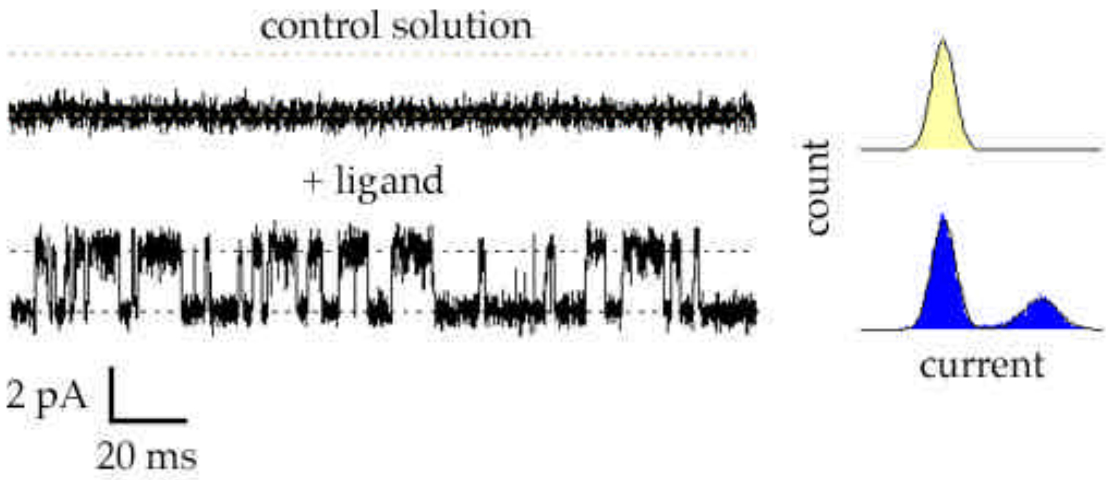

Figure 1: Single channel currents from a cGMP-gated channel. Histograms of the current flowing through the channel are shown at the right.

rate, our responses to hormones, and our senses of taste, smell and sight.

Bertil Hille's lab is interested in how the activity of ion channels is controlled by intracellular signals. Much of the work in the Hille lab centers on a particularly important family of signaling networks known as G-protein cascades. Although the receptor proteins in this family are all similar, a diverse set of mechanisms couple receptor activation to its eventual targets (in many cases ion channels). In some cases the coupling takes place entirely by substances diffusing on the cell membrane, while in others the coupling requires diffusion of a messenger through the cell's cytoplasm. Relating the properties of these different signaling cascades to physiological function provides an opportunity to uncover general rules governing their behavior.

Intracellular signaling plays a central role in muscle, where a small influx of calcium ions across the cell membrane is amplified by release of calcium from intracellular stores. The resulting rise in calcium triggers contraction of the muscle cells. Work in Luis Fernando Santana's lab focuses on how local and global Ca^{2+} signals control contraction and gene expression in

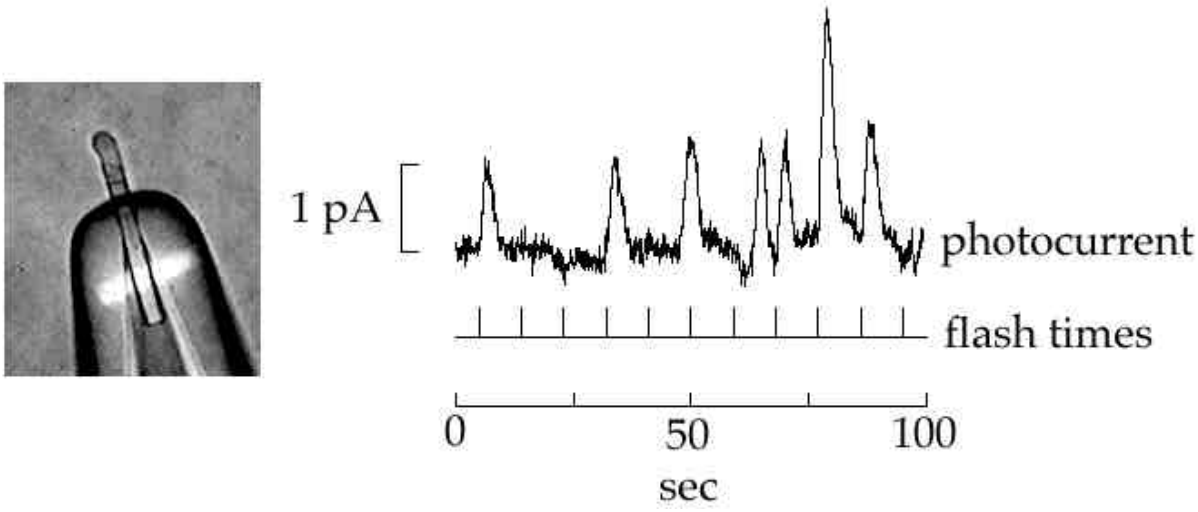

Figure 2: Suction electrode recording (left) of single photon responses in a rod photoreceptor.

muscle. This work involves a combination of molecular biology, confocal microscopy and electrophysiology.

Rod photoreceptors provide a striking example of the power of intracellular signaling. These cells are capable of detecting single absorbed photons because of amplification of the signal from a single active molecule by a second messenger cascade (Fig. 2). Indeed in many ways the performance of the rod photoreceptors compares favorably with that of the best man-made light detectors. Work in Fred Rieke's lab investigates how the rods reach this exquisite sensitivity and how the retina reads out the rod's minute signals.

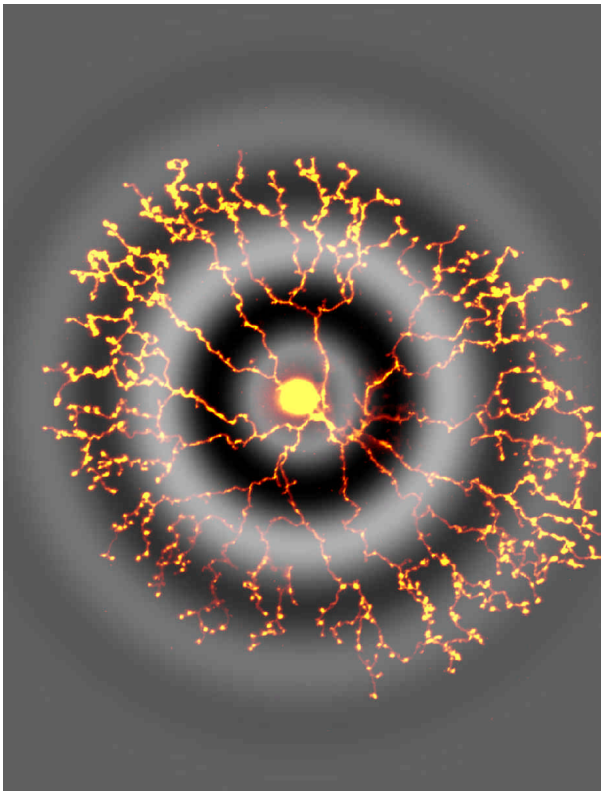

Figure 3: Two-photon image of a starburst amacrine cell filled with a calcium-sensitive dye. Calcium signals in the tips of the cell's processes show selectivity to the direction of a moving stimulus.

Kevin Conley's and Marty Kushmerick's labs are involved in related research, using spectroscopic methods to follow energy metabolism in living organisms in real time.

Neuronal biophysics

Computations within the nervous system are performed by single neurons and interactions between neurons. The properties of single neurons and communication between neurons in small neural circuits are essential components of the computational and signal-processing strategies used by the nervous system as a whole.

The retina provides an excellent opportunity to relate cellular events to visual function. Peter Detwiler, together with Winfried Denk, has pioneered the use of two-photon microscopy to study how retinal cells work. This technique provides a unique window on events in parts of cells and in cell populations as it permits fluorescence imaging while maintaining the retina's light sensitivity (Fig. 3). The Detwiler lab is using two-photon microscopy to understand how retinal cells compute based on their photoreceptor inputs. One example is how retinal cells generate directionally selective signals to moving objects. These experiments provide an excellent opportunity to relate cellular mechanisms to clear functional roles.

Research in the labs of Albert Berger, Marc Binder, Ed Rubel and Bill Spain also focuses on the computational properties of single cells and small neural circuits.

Systems and computational biophysics

All research in biophysics aims to understand the physical basis of the remarkable behavior of living systems. Several labs in the department attack this problem directly by measuring the electrical signals from single neurons or small neural populations in the brain of an awake, behaving animal. These experiments allow direct correlation of the properties of a single neuron or small sets of neurons with behavior.

How are sensory inputs transformed into signals appropriate to guide behavior? This is the central question of research in Mike Shadlen's lab. The responses of cells early in the visual system are tightly coupled to the sensory inputs – e.g. visual stimuli. Responses of cells near the motor output end – e.g. the motor neurons controlling muscles

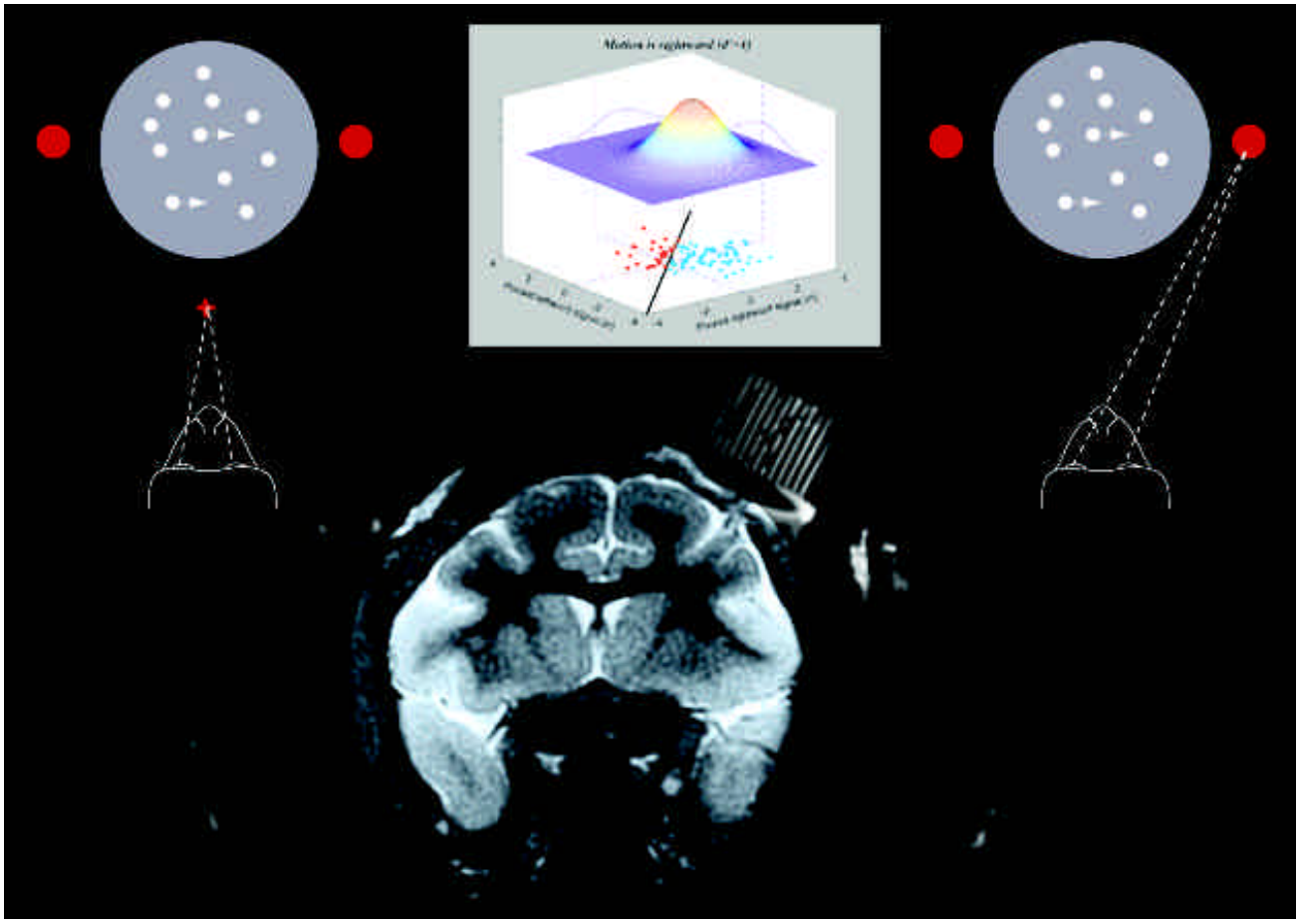

Figure 4: Probing the neural basis of decisions. The animal makes a decision about the whether the stimulus is moving to the right or left, and indicates the decision by making an eye movement. This decision can be compared with the electrical activity of neurons in the brain

– reflect the planned movement rather than the sensory input. What about cells in between? By recording electrical activity in cells exhibiting both sensory and motor characteristics, the Shadlen lab explores how evidence based on sensory information is accumulated to form the basis of behavioral decisions (Fig. 4). This work combines quantitative electrophysiology with statistical models of the decision process itself.

Work in labs of Marge Anderson, Eb Fetz, Albert Fuchs and Bharathi Jagadeesh also investigates the neural basis of behavior.

Theoretical biophysics

A strength of the department is quantitative experimentation. Our newest faculty member, Adrienne Fairhall, will add a theoretical perspective to work in the department. Her work focuses on rules for computation in neurons and neural circuits. One issue of particular interest is how neurons adapt to maintain sensitivity in the face of changes in the statistics of their input signals. This theoretical work leads to direct, experimentally testable, predictions about the dynamics of neural responses.

The areas highlighted above are a small subset of the biophysics research in the department. For more information, particularly about research areas not mentioned above, please see our departmental web site (http://depts.washington.edu/pbiopage/).

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Feature

An Interview With Larry Cohen

Sonya Bahar

Development of new imaging techniques is one of the most vibrant areas of contemporary biophysics. Dye imaging is one of the most beautiful, and useful, of these new research areas. Flip through the Molecular Probes catalog (or browse through it online at http://www.probes.com), and you will be awed by the varieties of dyes which have been developed just within the last few decades. Foremost among these are the voltage sensitive dyes (VSDs), a perfect tool for the electrophysiologist who craves spatiotemporal data. A major innovation, these dyes fluoresce proportional to the change in a cell's membrane potential. They are now a major tool of the trade for biophysicists studying cardiac electrophysiology and neurophysiology. But developing these probes was far from easy. Larry Cohen, of the Department of Cellular and Molecular Physiology at Yale University, was – and still is – one of the major figures in the development of this field. THE BIOLOGICAL PHYSICIST *talks with him about what it was like to be "present at the creation".*

Sonya Bahar: Where did the idea of fluorescent dye imaging originate?

Larry Cohen: For me this idea originated in 1967 at the Marine Biological Laboratory in Plymouth England where Richard Keynes and I were measuring intrinsic optical signals from squid giant axons. I was at dinner one night with Trevor Shaw and David Gilbert when David said: "You know these optical signals could be used to follow activity in the brain." The next night I asked him if he wanted to work on that idea. David said: "No". I have devoted much of my career to pursuing David's idea.

At that time the optical signals were tiny; 10,000 trials had to be averaged to measure them in a squid axon. David's suggestion that they could be used as a monitoring signal was really imaginative. Sadly, he died of the flu at age 39.

SB: What were the first voltage sensitive dyes like? Who synthesized them?

LC: The answer is a bit complex. The signals that Richard and I studied in 1967 were signals from intrinsic optical properties of axons; light scattering and birefringence. The next year Ichiji Tasaki and co-workers found that you could stain nerves with dyes and the dye fluorescence changed when the nerve was stimulated. However, Ichiji concluded that the signals were not voltage sensitive. Vicencio Davila, Brian Salzberg and I later (1970-74) asserted that those dye signals *were* voltage sensitive and an argument persisted in the literature for several years. The argument has been resolved in the sense that the community accepts these dye signals as voltage dependent.

Tasaki started with histological stains. You buy them from a catalogue. We tested about 150 different histological dyes and found one that had signals that were 5 times larger than those studied by Tasaki. Later we tested several hundred textile dyes but without much improvement. The breakthrough came from one of four dyes that were given to us by Eastman Kodak. This dye (Merocyanine 540) had a signal that was 25 times better than Ichiji's. We were then joined by a chemist, Alan Waggoner, who took the merocyanine dye from Kodak and started making derivatives. Together with his students C. H. Wang and Ravender Gupta, hundreds of derivatives were synthesized.

Some of these had signals that were 100 times larger than the originals and, in addition, they were 100 times less phototoxic. These dyes were good enough to be used as activity monitors.

SB: What led you to decide to undertake such a massive search for good voltage sensitive dyes?

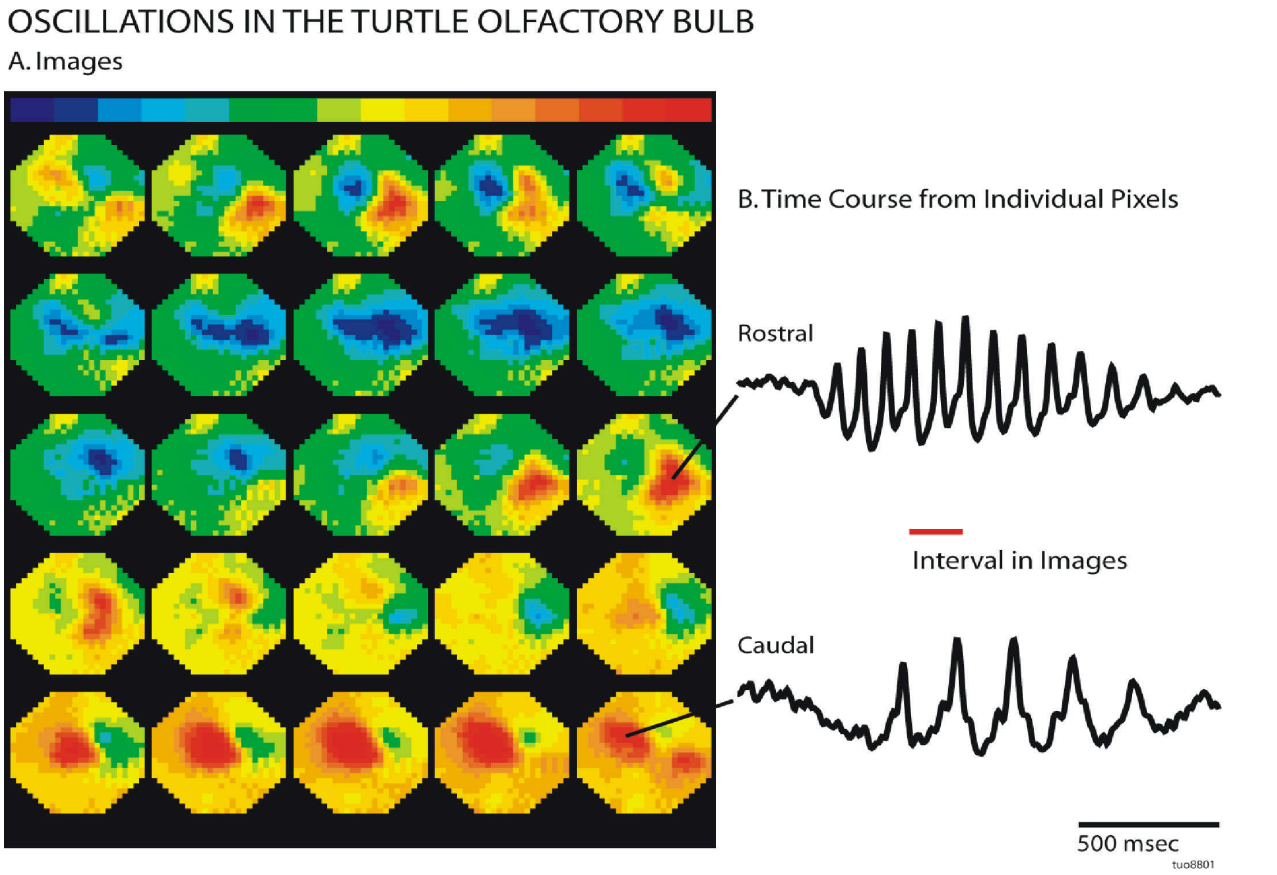
LC: At the beginning it was clear that increasing the numbers of tested dyes helped. After testing

100 dyes we had signals that were five times bigger than those used by Tasaki. The next 100 resulted in another improvement of a factor of 5. The next 500 resulted in yet another factor of 5. However, the next 1000 didn't improve signal size and we eventually gave up on the possibility of finding bigger signals. Much of the more recent synthesis has been designed to find dyes that work at longer wavelengths (to avoid blood vessel artifacts) or dyes that work better in particular preparations.

The early screening was carried out together with Brian Salzberg (Physics, Harvard), Bill Ross (Physics, Columbia), Amiram Grinvald (Chemical Physics, Weizmann) and Kohtaro Kamino. Some of us ran marathons together; clearly, we had personalities suited to long-term efforts. Indeed, Amiram and Rena Hildesheim have continued to synthesize new voltage sensitive dyes. The other lab still involved in this effort is that of Leslie Loew (Chemistry, Cornell) at the University of Connecticut Health Center.

SB: Why did you choose to focus on VSDs rather than calcium-sensitive dyes, for example?

LC: Just happenstance. You need both kinds of information; what is happening to membrane potential and what is happening with intracellular calcium, one of the important intracellular second messengers. While we claim the first paper using a dye (Arsenazo III) to measure intracellular calcium (Brown et al, 1975), clearly, Roger Tsien



Oscillations in the turtle olfactory bulb elicited by odors and imaged with a voltage-sensitive dye. Two different oscillations are shown, one rostral (anterior) and one more caudal (posterior). The rostral oscillation has a frequency that is 2.0 times higher than the caudal oscillation. On the right are times courses of the optical signals from individual pixels over a period of about 1.5 seconds. On the left are pseudocolor images of the oscillations that include two rostral events and one caudal. The time interval in the images is indicated by the red bar on the right. Both oscillations appear to be propagating waves. The time interval between frames is 8 msec. These measurements were carried out together with Ying-wan Lam, Michal Zochowski (Physics, Warsaw) and Matt Wachowiak.

and his collaborators have made the major contributions to this field.

SB: Describe what your lab was like in 1971. What was the atmosphere like? What synthesis tools did you have available? What sort of overall plan did you have for the project? Did you pick one type of dye and synthesize a number of variants, test them all, and then proceed to variants of another basic structure?

LC: My lab didn't do any of the synthesis; at that time it was all done in the Waggoner lab at Amherst College. The atmosphere was great; the four of us (Vicencio, Davila, Brian, Alan, and I) were young and enthusiastic.

There was no plan. We tried to learn from what worked and to make derivatives that might work better. In my view this area is an ugly case of a parameter space that is too large for a small group of scientists. Many parameters will affect how well a dye does in reporting membrane potential on a biological preparation including hydrophobicity, intrinsic voltage sensitivity, Stokes shift, pharmacological effects, phototoxicity, etc. Furthermore, there are probably 200,000 dyes already in existence and a nearly infinite number of dyes that might be synthesized. In the end, we tested less than 1% of the existing dyes. We have no idea whether we found the best available dyes or only got stuck in a local maximum. In contrast, Eastman Kodak was more serious. They needed better dyes to photosensitize silver grains for wavelengths longer than 450 nm. Utilizing a substantial fraction of the nation's organic chemists, Kodak synthesized more than 50,000 dyes in that effort.

SB: Did you have a sort of assembly line or did everyone in the group have a hand in all aspects of the project?

LC: We all contributed to all phases of the experiments that were done in the lab. This is part of what made it fun.

SB: How did you test each dye after it was synthesized? What sort of imaging system did you use?

LC: We tested both the absorption and the fluorescence signal for each dye using the squid giant axon. This preparation was nearly ideal because you could measure membrane potential, ionic current, and optical signal simultaneously from the same, relatively large, patch of membrane. In the transmitted light path (absorption) we formed an image of the axon on a photodiode and used slits to block the light that didn't go through the axon. The fluorescence path was simpler; a secondary filter to block the incident wavelengths and large photodiode was positioned close to the axon at 90^{o} to the incident light.

We could test about 200 dyes on 200 axons per summer at the Marine Biological Laboratory in Woods Hole, USA. Good dyes needed several axons; on the other hand, we would often use only one axon to test three or four dyes that gave no signal.

SB: Were there times when you thought the search would never bear fruit?

LC: Not really.

SB: What was the initial reaction of the scientific community to this type of imaging approach? Were your publications snapped up immediately by journal editors, or did you have to fight with reviewers to convince them that the signals you were measuring were real?

LC: The initial reaction was very supportive and positive. The data we generated was convincing so there was no problem getting it published. Like everyone else, we had papers which should have been accepted by Nature but weren't (the calcium dye paper in 1975) and other papers that were accepted by Nature that probably shouldn't have been.

SB: Has the field progressed faster or more slowly than you expected?

LC: More slowly. But not so slowly that the progress hasn't been gratifying to me and other members of the community.

SB: How many labs would you estimate are using VSDs today?

LC: About 100.

SB: Are there any major new advances around the corner, such as big increases in fluorescence intensity from new dyes, decreases

in phototoxicity, or increases in spatial resolution?

LC: There are big advances coming in cellular specificity of staining. These advances will come from genetically engineered animals that express protein sensors (or enzymes capable of generating sensors) in individual neuron types. For example, you will be able to stain and record independently from both the input and the output neurons of a brain area and thereby determine the input-output transfer function. This would be a big step in understanding the brain.

A second big advance in spatial resolution is already occurring. Fast (>1000 fps) high resolution CCD cameras are now available. At present they have a limited well depth (about 300,000 electrons) but I expect that the well-depth will increase by a factor of 100 in the near future. Deeper wells mean improved signal-to-noise ratios in fluorescence and absorption measurements where there is a relatively high light intensity.

SB: How well is the signal transduction mechanism of VSDs understood today?

LC: In a general way the transduction mechanism is understood for some dyes. We can say, for example, that the Merocyanine 540 dye signal arises from a voltage dependent shift in the monomer-dimer equilibrium. On the other hand, we have not learned enough about the mechanisms to direct the synthesis of new dyes with markedly bigger signals.

SB: What are your feelings about your scientific career?

LC: I feel lucky that I was successful in meeting the early scientific goals and grateful for the continued personal support from my collaborators and the community. In addition, it has been very gratifying that clinically relevant results in epilepsy and cardiac arrhythmias have been obtained with these methods.

For more information and some amazing images taken with VSDs, check out http://www.redshirtimaging.com/. For more details about current research projects in Larry Cohen's laboratory at Yale, see http://info.med.yale.edu/cmphysiol/cohen/redshirtdiaries.html

Selected Bibliography – Recent Papers:

Gray RA, Pertsov AM, Jalife J. Spatial and temporal organization during cardiac fibrillation. *Nature* **392**(6671): 75-8, 1998.

Witkowski FX, Leon LJ, Penkoske PA, Giles WR, Spano ML, Ditto WL, Winfree AT. Spatiotemporal evolution of ventricular fibrillation. *Nature* **392**(6671): 78-82, 1998.

Choi BR, Nho W, Liu T, Salama G. Life span of ventricular fibrillation frequencies. *Circ Res* **91**(4):339-45, 2002.

Momose-Sato Y, Sato K, Kamino K. Optical approaches to embryonic development of neural functions in the brainstem. *Prog Neurobiol* **63**:151-97, 2001.

Demir R, Haberly LB, Jackson MB. Imaging epileptiform discharges in slices of piriform cortex with voltage-sensitive fluorescent dyes. *Ann N Y Acad Sci* **911**:404-17, 2000.

Selected Background Papers and Reviews:

Zochowski M, Wachowiak M, Falk CX, Cohen LB, Lam YW, Antic S, Zecevic D. Imaging membrane potential with voltage-sensitive dyes. *Biol Bull* **198**(1):1-21, 2000 (Review).

Wu JY, Lam YW, Falk CX, Cohen LB, Fang J, Loew L, Prechtl JC, Kleinfeld D, Tsau Y. Voltage-sensitive dyes for monitoring multineuronal activity in the intact central nervous system. *Histochem J* 1998 Mar;30(3):169-87, 1998 (Review).

Orbach HS, Cohen LB, Grinvald A. Optical mapping of electrical activity in rat somatosensory and visual cortex. *J Neurosci* **5**(7):1886-95, 1985.

Loew LM, Cohen LB, Salzberg BM, Obaid AL, Bezanilla F. Charge-shift probes of membrane potential. Characterization of aminostyrylpyridinium dyes on the squid giant axon. *Biophys J* **47**(1):71-7, 1985.

Cohen LB, Salzberg BM, Grinvald A. Optical methods for monitoring neuron activity. *Annu Rev Neurosci* **1**:171-82, 1978 (Review).

Ross WN, Salzberg BM, Cohen LB, Davila HV. A large change in dye absorption during the action potential. *Biophys J* **14**(12):983-6, 1974.

Cohen LB, Keynes RD. Changes in light scattering associated with the action potential in crab nerves. *J Physiol* **212**(1):259-75, 1971.