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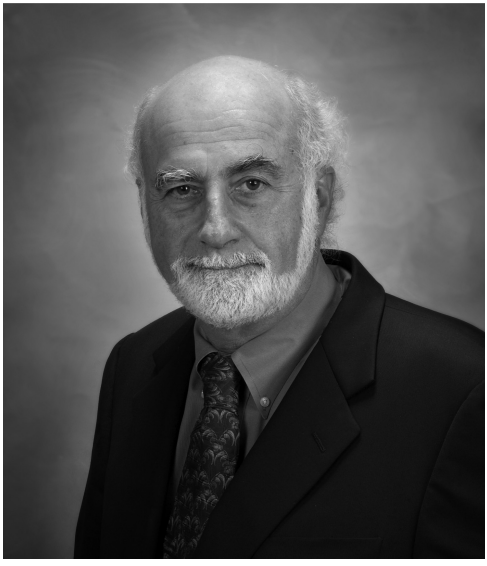

John H. Byrne

Born:

Englewood, New Jersey
February 15, 1946

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New York University Tandon School of Engineering, BS (Electrical Engineering, 1968)
New York University Tandon School of Engineering, MS (Bioengineering, 1970)
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Assistant to Associate Professor, Department of Physiology, School of Medicine, University of Pittsburgh (1976–1981, 1981–1982)
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NIH Research Career Development Award (1978)
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President's Scholar Award for Research, The University of Texas Health Science Center at Houston (1998)
Fellow, American Association for the Advancement of Science (2001)
Hebb Award, International Neural Network Society (2004)
President's Award for Mentoring Women, UT Health Science Center at Houston (2006)
Award for Education in Neuroscience, Society for Neuroscience (2007)
President, Association of Medical School Neuroscience Chairpersons (2008–2009)
Innovations in Health Science Education Award, The University of Texas System (2012)
President's Scholar Award for Teaching, UT Health Science Center at Houston (2014)
Member, Alpha Omega Alpha Honor Society (2017)
The University of Texas System Regents' Outstanding Teaching Award (2017)

John Byrne's work has focused on the understanding of the cellular and molecular mechanisms underlying behaviors and their ability to be modified by learning. He has done so by using a combination of biophysical, biochemical, molecular, and mathematical modeling techniques. His work on operant conditioning is particularly significant because it constitutes the first detailed mechanistic analysis of this major form of associative learning and provides the basis for a comparative analysis of the mechanistic interrelationships among different forms of learning. Another novel aspect of his research program is his pioneering use of complementary empirical and mathematical modeling approaches to the understanding of memory. In addition to his research, Byrne has taken a leadership role in the community of neuroscientists and has organized and participated in outreach activities at the local and national levels. He is also a dedicated and innovative educator. He is an author or editor of 15 books, including a graduate text on cellular and molecular neuroscience. In addition, he is the developer of an open-access web-based neuroscience electronic textbook.

John H. Byrne

John H. "Jack" Byrne

It is a great honor to write an autobiography for this series. Perhaps my story will resonate with others and provide some insight and guidance for their own careers. Perhaps also this chapter will provide some insight into an era during which great progress was made in understanding mechanisms of learning and memory by exploiting the technical advantages of invertebrate model systems. So, like most autobiographies, let's start this voyage at the beginning. But if you choose not to read further, here is the bottom line. The extent to which I have had a successful career in brain science is due to having some brains, putting in hard work, having great mentors and collaborators, choosing the right boat, being in the right place at the right time, having a supportive spouse and family, and having other good luck.

When I was a boy, my father told me of his harrowing experiences dodging U-boat torpedoes as a captain of a Liberty ship transporting cargo to England during World War II. It is my good fortune that he made it home. He chose the right boat, as many others on those convoys were less fortunate. Pop (as we called him), John James Byrne (b. 1915), was the son of Charles John Byrne (b. 1876), a native of Dublin, Ireland. Charles worked in New York City as a poultry inspector. Tragedy struck when he died of a heart attack in a doctor's office in 1935, just two weeks after he canceled his life insurance policy. His (and my) good fortune was marrying my grandmother, Roseanna Blanchfield, in 1908, who was also born in Dublin (b. 1878). Charles and Rosanna had six children; my father was the fifth. After high school, Pop pursued a maritime career, first working as captain of charter fishing boats out of Sheepshead Bay, New York, as a tugboat captain in New York Harbor, and then as a captain of Liberty ships.

My mother, Lillian, was born in 1914 on a houseboat on the Hudson River just south of the George Washington Bridge on the New Jersey side. She was one of the five children of Katherine (Winkler, b. 1886) Schwartzman and Frank Schwartzman (b. 1884). The Winklers lived in Moravia, now part of the Czech Republic. In 1885, they emigrated to the United States and established a dairy farm in New Jersey, where my grandmother Katherine Winkler was born. The Schwartzman family emigrated to the United States in 1852 from Bavaria after the family mill was destroyed by fire. Grandfather Frank Schwartzman was a successful fishing boat captain and also reportedly had a side business as a "rum runner" during Prohibition. He owned several fishing boats operating out of New York Harbor in the 1920s and 1930s, including the 189-foot steam fishing yacht *Colonia*. On one fishing trip, Frank Schwartzman and the *Colonia* rescued 34 men from a vessel

at the point of foundering off of Coney Island (*New York Times,* October 21, 1930). Frank Schwartzman died of a brain tumor in 1937. His two sons continued the maritime tradition with son Frank becoming an oil tanker captain and Howard becoming a ship pilot on the Panama Canal.

Boats and the sea were a common thread in the early lives of my father and mother. Indeed, John James Byrne and Lillian Schwartzman met on a fishing boat. They were married in 1943. After World War II, my father retired from the Merchant Marine to raise a family, and the couple moved to Freeport, a small town on the south coast of Long Island about 30 miles from New York City. My father also began a new career as an electrical contractor and started the Byrne Electric Company.

I was a baby boomer (b. 1946) and the second of six children. At Holy Redeemer elementary school, I was not particularly concerned about high grades; Bs, or even Cs were fine. My priority was to have as little to do with schooling as possible, and at the same time work as much as I could on odd jobs, such as mowing lawns, raking leaves, shoveling snow, and delivering newspapers and magazines. Work was a bit of a necessity, because there were times when customers of Byrne Electric failed to pay their invoices and sometimes things got rather tight. It was ironic that our home electricity was once turned off by the power company because we failed to pay our electric bills. So, to get anything extra, you were on your own. I did contribute to the family by helping my father on the job. I was mostly a gofer and someone who held the flashlight, but I learned something about electricity and electrical circuits. At a young age, I also had an interest in learning how things worked. I would disassemble bicycle backpedal brakes, lawn mower and outboard motors, and old vacuum tube radios to see what was inside and discover how they worked. In retrospect, that was the beginning of a trajectory of discovery ultimately leading me to science.

During elementary school, my parents both became interested in amateur radio, and they dragged me and my older sister along. We both learned Morse code and radio theory necessary to pass the Federal Communications Commission (FCC) exam for General Class amateur radio operator licenses. That experience allowed me to learn more about electronics and the principles of voltage, current, and resistance. (Little did I know that I was also learning about basic principles of heat transfer, solute diffusion, and fluid flow.) In those days, amateur radio enthusiasts built much of their own equipment. I built a transmitter from scratch and used it to communicate with other "hams" around the United States and the world.

Parochial school went only to the eighth grade, so by then, I needed to decide whether to go to a parochial high school or enter the public-school system. It was an easy decision for me. I had had enough of the dogma, rigidity, and occasional physical abuse. (A nun once pulled me down to the floor by my ear to punish me for something I did not do.) And then there was the issue of the Holy Ghost, a concept I could never understand.

At Freeport Junior High School, I was exposed to two inspiring teachers, one in algebra and the other in social studies. I excelled in those courses and enjoyed getting good grades. When it was time to move on to 10th grade at Freeport High School, I was pleased to learn that my teachers had recommended me for the honors program. Here, I had the opportunity to continue to enjoy learning in an environment with a group of other highly motivated students. Looking back, I was extremely fortunate to have had the opportunity to be exposed to such an enriched academic environment. I regret that so many students are less fortunate. Public schools in Long Island, like those in Freeport and nearby Baldwin and Merrick, were excellent and included a diverse body of students. Some Freeporters in that era who went on to successful careers in the arts and sciences included Carolyn Boriss-Krimsky (my home roommate in high school), Arthur Davidson, Lou Reed, and Harold Varmus. Freeport High School was fertile with opportunities. I joined the Mask and Wig Club, eventually becoming technical director of the high school senior variety show. I joined the math club and eventually became its president. I cofounded the high school amateur radio club and became its president. The faculty adviser for the radio club was Mr. Parker, who was also in charge of the Industrial Arts program. He would be frequently contacted by outside companies looking for help from high school students. He told me about a summer job at Columbian Bronze Company, a firm that was known for its ship propeller manufacturing but that had begun to market marine radio telephones and depth finders. They had a need for a student with an electronics background who could troubleshoot and repair defective devices.

It was on to college after the summer job at Columbian Bronze. My background with electronics naturally led me to electrical engineering. I was not particularly interested in going to an away school, so I looked for local schools. One that attracted me was the Polytechnic Institute of Brooklyn (Poly, now New York University Tandon School of Engineering). I became a commuter; taking the Long Island Rail Road back and forth between Freeport and Brooklyn. Poly had great strengths as an engineering school, but socializing was limited because most of the students were commuters, and it was as far from a party school as you could get. Indeed, there was only a single female freshman in my entering class. So all socializing was local. Fortunately, there were several other commuter types like me from Freeport who attended local colleges like Adelphi and Hofstra Universities and some friends from high school who had local jobs. We frequented local bars and clubs and dated some of the high school seniors. That's where I met Susan. We went out during her senior year and that summer. We went our separate ways, but four years later got back together and have been together ever since. The successes that I describe here would not have been possible without her love and support.

My expertise with radio telephone repair at Columbian Bronze led me to a job repairing radio telephones with American Hydrofoil Lines during

the summer of 1964. American Hydrofoil ran hydrofoil boats for commuters between Port Washington, Long Island, and Wall Street, and passengers between 34th Street and the 1964 World's Fair in Flushing, Queens. Sometimes, I filled in as a deckhand. On one occasion, I worked on a private charter to take Richard Nixon to the World's Fair. That was my first interaction, albeit minimal, with a celebrity. In the summer of 1965, I landed a job with the Computer Simulation Laboratory at the U.S. Naval Training Device Center in Sands Point, Long Island. There, I was exposed to large-scale analog computer simulations of ship docking and helicopter flight. The unit was being relocated to Orlando, Florida, but my boss John Cavallari opted not to move and instead accepted a position as director of the electronics lab in the department of medical physics at Memorial Sloan-Kettering Cancer Center in New York City. He invited me to work with him again the following summer. Working at Memorial was a great experience, because I was exposed to the field of biomedical instrumentation. Working in a clinical setting also exposed me to medicine and the realization that so little was known about treatments and cures for many diseases. I have not forgotten the sadness I felt when seeing the hairless young children receiving radiation treatment on the machines we maintained. I was also becoming interested in neuroscience, because my mother was diagnosed with multiple sclerosis. It was discouraging to learn how little was known about its cause, mechanisms, and effective treatments.

I did not realize it at the time, but my hard-work ethics, electronics background, and life experiences was moving me toward a career in biomedical sciences. I just needed some direction. A chance encounter with a Poly student whom I had worked with at the Naval Training Device Center the previous summer set me on the trajectory of a life in neuroscience. While waiting for an elevator one day in the fall of 1966, he told me about a part-time job for an electronics technician, which was available at NYU Medical School. I called the number he gave me and spoke with Eric Kandel. Kandel invited me for an interview and offered me the job. Needless to say, it turned out to be more than a job; it was a compass pointing to the future.

NYU and the Golden Age of *Aplysia*

Eric Kandel had recently set up his lab at NYU after moving from Harvard University. Kandel's vision was to exploit the technical advantages of the marine mollusk *Aplysia* to elucidate mechanisms of learning and memory. Those advantages included simple stereotyped behaviors, a nervous system with only about 20,000 neurons, and neurons that were extraordinarily large compared with vertebrates. The Kandel lab consisted of an assistant professor, Irving Kupfermann; one postdoc, Harold Pinsker; and two graduate students, Daniel Gardner and Howard Wachtel. Irving was trained as a behavioral psychologist. He had worked as a postdoc with Eric at Harvard

on the original description of the electrophysiological properties and synaptic connections of neurons in the abdominal ganglion of *Aplysia*, in a classic series of papers. He had Coke-bottle-thick eyeglasses. He looked smart, and he was. By his appearance you would mistake him for a beatnik in Greenwich Village. And then there was Harry Pinsker. If Irving was a beatnik, Harry was a hippie. Harry came as a postdoc having received his doctorate in psychology from the University of California, Berkeley. At Berkeley, he was part of the political activism of that time. But despite politics, he was highly engaged in the research and was a driving force in studying the learning capabilities of *Aplysia*. The lab also included a graphic artist, Kathrin Hilten, as well as a technician and a secretary. This original group was soon expanded to include Jim Blankenship, who did his thesis on synaptic potentials in cat spinal motor neurons; Vince Castellucci, who did his thesis on electrophysiological recordings from cat glial cells; and Tom Carew, who did his thesis on cortical spreading depression. Adjacent to the Kandel lab was the lab of Alden Spencer, whom Eric helped recruit to NYU. They had previously worked together at the National Institutes of Health (NIH) on the now-classic study of intracellular recordings from the hippocampus of awake animals. Alden's lab consisted of Bob April, a neurology fellow; Bob Leibovitz, a postdoc; Esther Gardner, a graduate student; medical doctor/doctoral students Marc Dichter and Micki Selzer; and a technician.

My job was to repair equipment and design and build special equipment. Harry Pinsker in particular was always looking for new gadgets for his electrophysiology rig, so I was kept busy with projects. It was an exciting time to be in that environment. The Carew, Castellucci, Kupfermann, Pinsker, and Kandel team were doing the now-classic studies of the synaptic mechanisms of habituation and dishabituation. The idea that synaptic plasticity is a mechanism of memory had been around since Ramón y Cajal and Eugenio Tanzi. Kandel's group went to the next level by showing that it actually occurred. They recorded decreases and increases in synaptic strength between sensory neurons and motor neurons mediating the gill-withdrawal reflex during habituation and sensitization, respectively. The intellectual environment was exciting and diverse with broad representation of cellular neurophysiologists and psychologists, and later biochemists when Jimmy Schwartz began collaborating with the group. I was exposed to the new and intriguing language of neuroscience. But it was verbal whiplash with so many new terms and each one with a worthy complement. Imagine a young man having virtually no knowledge of neuroscience, or even biology, being exposed to constant chatter about excitation, inhibition, disinhibition, feedforward inhibition and feedback inhibition, excitatory and inhibitory postsynaptic potential (EPSPs and IPSPs), homosynaptic and heterosynaptic plasticity, antidromic and orthodromic spikes, habituation and dishabituation, and associative and nonassociative learning. And there were constant references to a magical neuron called Interneuron II, which

had widespread effects on numerous neurons in the abdominal ganglion, magical because the cell had not been identified despite years of searching. I had had no coursework in biology, but I began to assimilate some knowledge of neuroscience. Socially, this was a great group of very smart people excited about what they were doing. There were always great discussions at lunch in the NYU cafeteria about science, scientists, and biological and national politics. For the first time, I had a new appreciation for science and the people who do it. And unlike the stereotype of nerdy scientists, these folks led normal lives and were super friendly, open, and of good humor. One year at the holiday gift exchange, my gift was a children's electricity project. Irv Kupfermann, who was using von Frey hairs to deliver tactile stimuli to the animal's skin, received an envelope containing a clip of what appeared to be pubic hairs, which presumably could be used as novel tactile stimulators. We all had a big laugh.

Prominent scientists often visited the lab. A real treat was being introduced to John Eccles and John Nicholls, for me an entirely new class of celebrities. They all had interesting personalities and good humor. Alden Spencer told us after Eccles's visit that the Nobel Laureate had said that "the problem with brain research is that the people doing it have none." Eccles was obviously not referring to the Kandel group. The lab was making breakthrough discoveries, and the scientific world saw *Aplysia* as a model system to help demystify mechanisms of learning and memory. Kandel was a young superstar. Always exuberant, Vince Castellucci said Eric was god and this was the "golden age of *Aplysia*." In a small way, I felt that I was part of that age.

There was another overlapping influence, the Vietnam War and the student protests that exploded across the country. I was part of it to a certain extent, attending peace rallies in Central Park and Washington, D.C. Harry Pinsker was engaged at another level. He was a committed antiwar activist and a leader of the strike at NYU Medical School. They took over the print shop and used it to print antiwar material. Harry's extensive outside activities did not go well with Eric, however. Time away from research for any reason was unacceptable, and I sensed that serious damage was done to their relationship.

Should I Stay or Should I Go?

As a senior in college, when spring came, it was time to start looking for jobs. My attention was drawn to a posting on the Poly job board for a bio-instrumentation engineer at Grumman Aircraft Company in Bethpage, Long Island. I applied for that job, received an offer, and accepted the position. It was well paying and an opportunity to work on bio-instrumentation for the lunar lander that was being built by Grumman. So, it seemed that my brief but spectacular time at NYU was ending. But when I told Eric about the job and that I would be leaving the lab, he suggested that I should

"continue working at the lab at nights and on weekends." It may seem crazy to work two jobs, but working at NYU did not seem like work. It was a good deal for Eric to retain an experienced electronics person and a good deal for me to continue supporting a great group of scientists working on exciting projects and being part of the "golden age." But it turned out to be more than just fixing equipment and designing devices. I had a deal with Harry Pinsker: after finishing his experiment, he would leave his isolated ganglion on the rig for me to experiment with if I agreed to clean things up at the end. (He trusted me with his rig because he knew I would fix it if I broke anything.) So, I started making microelectrodes and poking cells. It was a fantastic thrill to impale my first neuron and see real action potentials, synaptic potentials and patterns of neuronal activity in a living brain. I began to think that I could be a neuroscientist like the graduate students and postdocs in the lab. I was inching closer to a career in neuroscience.

That spring I was also notified by the student counselor at Poly that I was two credits short of graduating. Oops! How that happened is lost in time, but it was a chance to make some lemonade from a lemon. The lemonade was taking a course in biopsychology, which provided the needed two credits, but importantly, also provided an opportunity to formally begin learning about the nervous system and behavior. I am embarrassed to say, but I felt a bit superior to the other students in the class when we learned about action potentials and synaptic transmission. Those dynamic electrophysiological processes were static images in the textbook, but I had seen them and recorded them in a living neuron. So, in the fall of 1967 I was working a full-time job and traveling after work at Grumman in Bethpage to New York City to work in Kandel's lab and take the course on biopsychology.

Graduate School at NYU

I enjoyed my time working at Grumman. I had an opportunity to work with the astronauts, outfitting them with electrocardiogram (EKG) electrodes and carbon dioxide (CO_2) and oxygen (O_2) sensors for tests of the environmental control system in a mock lunar lander. I also worked on one of the first DEC PDP-8 computers. This experience led to an assignment at Cape Canaveral working on simulated launch sequences. The task was boring, but the environment was great. One day, I drove to the launch pad and walked under a Saturn V rocket. But in the spring of 1968, we were informed that our unit at Grumman would be relocated to the Johnson Space Center in Houston. I had no interest in relocating to Houston (seems ironic because I ended up there 14 years later anyway), so I decided to pursue my growing interest in neuroscience at graduate school. I was accepted in the Bioengineering Program at NYU in 1968 and was delighted to find out that I would receive a stipend of $3,000 per year from an NIH Training Grant. The stipend was special. For the first time, I was being paid to go to school.

My master's project was working with Alden Spencer and Esther Gardner on a project to design and build a feedback-controlled stimulator for the delivery of precise tactile stimuli to the foreleg of a cat while simultaneously recording single-unit responses in the somatosensory cortex. The project was successful, but I was increasingly attracted to the *Aplysia* work in Kandel's lab. For my doctoral project I proposed to Eric that I design an improved tactile stimulator and use it to study the mechanoreceptors in *Aplysia*. Here, I was inspired by the work of Vernon Mountcastle and Gerhard Werner quantifying the stimulus-response properties of mechanoreceptive cutaneous afferents, and the work of John Nicholls and Denis Baylor studying the receptive field and response properties of mechanoreceptors in the leech. I found that, with a single exception, all *Aplysia* mechanoreceptor neurons have similar thresholds and gave similar responses to vertically applied forces. The receptive fields of the mechanoreceptor neurons could be grouped into classes according to size and location on the skin. The boundaries of at least some receptive fields seem to be invariant from animal to animal. The project went well and resulted in a paper in the *Journal of Neurophysiology*. In my opinion, that paper still stands as the most quantitative analysis of molluscan mechanosensory neurons.

Introduction to Neuronal Modeling

I had known about the power of computer simulations of physical systems using linear differential equations from my summer job at the Naval Training Device Center. Through my graduate courses on systems analysis and readings, I became aware of the use of those approaches to analyze and model the dynamics of biological systems. I was particularly impressed with the scope of work presented in the published proceedings of a 1969 conference organized by Carlo Terzuolo on *Systems Analysis Approach to Neurophysiological Problems*. That inspired me to think that I could simulate the *Aplysia* nervous system by applying my math and electrical engineering background. I was a bit naïve to say the least, as this is an ambitious goal even today. I started modestly with a differential-equation based model of the dynamic response of the sensory neurons using the IBM simulation language Continuous System Modeling Program (CSMP). It was then that I came up against the reality that the nervous system was nonlinear and that the linear systems approach had limited utility. (I soon took a course on nonlinear systems.) I also realized that you can't model anything unless you have data. I had data on the sensory neuron responses, but I needed to go deeper into the system.

Lifelong Friends

During my time at NYU, I shared a rig with Vince Castellucci. He was the "dayshift" and I had the "night shift." I started my experiments at about five in the afternoon when Vince finished and commuted back to Port

Washington, Long Island, along with Tom Carew and Irv Kupfermann, who also lived there. I developed warm friendships with that crew as well as with Jim Blankenship, John Koester, and Kathy Hilten. We all had our scientific bond but, Irv, Tom, Vince, and I had a special bond because of our shared experiences on the Long Island Rail Road. Living in the same community made it easy to create a sense of extended family. All of these friends came together for Susan and my backyard wedding in Garden City, Long Island, in 1972. We were renting a house on the waterfront in Bellmore, Long Island, and had a small 15-foot motor boat. I have fond memories about digging for clams in the Great South Bay with Marcello Brunelli, Norbert Dieringer, and John Koester; water skiing with Mary Jo and Tom Carew and their family; and taking a trip to Gilgo Beach by boat with Lise and Vince Castellucci and Lise's mother. And then there were Dan and Esther Gardner, who I would meet every year at the meetings of the Society for Neuroscience (SfN). Eric and I also established a lifelong friendship. We would meet at least once a year at the SfN or on other occasions.

From NYU to Columbia

Quantitative Analysis of the Mechanoreceptor Neurons and Their Role in the Gill-Withdrawal Reflex

After graduating in 1973, I stayed on with Kandel as a postdoc so I could do a deeper dive into the circuitry of the withdrawal reflex. That transition coincided with a move of the lab from NYU to Columbia University. I was fortunate to receive an individual F32 postdoctoral fellowship from the NIH. I continued studying the sensory neurons but moved to an analysis of the stimulus-response properties of the sensory neurons and the quantitative contribution that the sensory neurons make to the gill-withdrawal reflex. My experimental preparation consisted of the isolated abdominal ganglion with the attached siphon and gill. I used my feedback-controlled stimulator to deliver controlled force probe stimuli to the siphon skin and a photocell to monitor gill withdrawal. These studies showed that the stimulus-response properties of the sensory neurons could be fit with simple algebraic functions and that the responses were stable to repeated stimulation. Moreover, the gill contractions produced by artificially firing motor neurons were stable. These later effects were significant because they indicated that neither of these peripheral sites could explain habituation of the reflex, thus supporting the role of depression of the sensory neuron synaptic connections in the central nervous system (CNS) as a mechanism for habituation. A second important question addressed during my postdoctoral work was the quantitative contribution that the mechanoreceptor neurons make to the reflex. I found that a single sensory neuron spike contributes about 7–36 percent of the motor neuron input produced by a weak probe stimulus; that

about 50 percent of the motor neuron input is monosynaptic; and that the depression of the sensory to motor neuron monosynaptic EPSP tracks the depression of the probe-evoked motor neuron synaptic input. These 1978 papers still stand as the most quantitative analysis of a molluscan reflex pathway. (These papers, and an independent one by Gordon Shepherd in 1978, were the first to introduce the term "microcircuit" into the neuroscience literature.)

One somewhat surprising observation was noted in the second of our two 1978 papers. In some cases, we observed EPSPs in the motor neurons when a probe was simply placed in the experimental chamber without touching the skin. However, we never encountered sensory neurons with such low thresholds for vibratory stimuli. We suggested that this observation may simply reflect a sampling bias, but it may also represent a previously undetected group of mechanoreceptor neurons that could also respond to waterjet and punctate tactile stimuli. Later studies by Robert Hawkins and Terry Walters provided evidence for the existence of such a low threshold population of sensory neurons, whose somata appear to be in the periphery. The take-home message was this: the interpretation of the results can be dependent on the specifics of the experimental conditions. For me, this take-home message was reinforced by the great central vs. peripheral controversy.

The Great Central vs. Peripheral Controversy

During that time, a major controversy developed between the Kandel lab and the labs of Jon Jacklet, Ken Lukowiak, and Bert Peretz on the role of the central and peripheral nervous systems in mediating the reflex. Kandel and Kupfermann in their 1969 *Science* paper, first characterizing the gill-withdrawal reflex, stated that the reflex is abolished when the abdominal ganglion was removed from an intact animal. Similarly, in my preparation of the isolated siphon, gill, and abdominal ganglion, the reflex was abolished when the nerves connecting the ganglion to the gill and siphon were severed. So, in our minds the critical role of the CNS in mediating the reflex was indisputable. Peretz and colleagues showed in a 1970 *Science* paper and several others that the reflex was intact after severing the nerves. The differences could not have been more striking! Contentious exchanges followed between the two groups at several meetings of the SfN. The neuroscience community was confused and intrigued. Who could you believe? Was the entire model of central neuron synaptic plasticity underlying habituation being torpedoed? Clearly, a resolution was needed. Eric invited Jacklet, Lukowiak, and Peretz to Columbia to try to resolve the issue. We would demonstrate our findings, and they would demonstrate theirs (although we doubted that they could). Perhaps differences in our experimental preparations might explain our different results. We monitored gill responses with a photocell, whereas, Peretz et al. used a tension transducer. We used either an 800-ms duration water jet

stimulus from a Waterpik® or an 800-ms duration feedback-controlled force punctate stimulus to the skin; Peretz et al. used a 30-ms duration punctate stimulus from a "tapper," a solenoid-driven metal rod. I suggested that we use my rig to run the two preparations simultaneously.

On the meeting day, we ran the two preparations simultaneously. Both the controlled-force stimulus and tapper stimulus produced nice responses from both the photocell and the tension transducer in the pretest. Then we cut the nerves. The controlled-force stimulus was delivered—no response, a result we expected. Next, the tapper stimulus was delivered. A response was observed, *unchanged from the pretest*. We were aghast. Both groups were correct! The difference seemed to be that the engagement of the central vs. peripheral nervous system was dependent, at least in part, on the nature of the stimulus. Lesson learned: When two groups have conflicting results, the differences may be explained by differences in the experimental protocols. A 1979 paper by Carew et al. summarized these and other findings related to the contributions of the central and peripheral nervous systems to the gill-withdrawal reflex.

University of Pittsburgh

One day Eric showed me a letter from Ernst "Ernie" Knobil, chair of the Department of Physiology at the University of Pittsburgh, inquiring whether Eric knew of any potential candidates for a junior faculty position in his department. (The "old-boy network" at work.) Eric encouraged me to apply knowing that Knobil was a first-rate scientist and academic leader and that the department had several distinguished faculty members, including Stanley Schultz, his former roommate during his first year at NYU Medical School. Schultz had become a leader in the field of membrane transport. Eric also knew of the work of Andre Borle who had found in 1974 that cyclic adenosine monophosphate (cAMP) could stimulate calcium efflux from mitochondria, a mechanism Eric thought might explain serotonin-induced synaptic facilitation in *Aplysia*. Gerhard Werner, whom I admired for his previous work on quantifying responses of cutaneous afferents, was also at Pittsburgh. So, Pittsburgh definitely had some strengths. But being a myopic New Yorker, Pittsburgh also seemed like a foreign country. My wife agreed. When I told her about the possibility of an interview, she said, "That's just for practice, right?" I visited Pittsburgh and was impressed with Ernie Knobil, the department, the stellar faculty, and the delightful city. I also had an offer from the University of Chicago. Eric wanted me to stay on, but I felt I needed to see if I could make it on my own. So, it was on to Pittsburgh, and I started the position on January 1, 1976, with a hard-money salary of $18,000 a year and a start-up package of $30,000. Those numbers seem rather chintzy by today's standards, but actually that package was very competitive. Also, my teaching responsibility was to be fairly

light, which, in retrospect, was important for providing time to develop my research program. That first year, I just had to give one lecture on temperature regulation for the physiology course. Ultimately, I took over the lectures on resting potentials, action potentials, and synaptic transmission. I also filled in for Stan Schultz, giving his membrane transport lectures while he was on sabbatical at the University of Cambridge. (Stan's and my lecture notes formed the basis of our book *An Introduction to Membrane Transport and Bioelectricity*.) I'll say more about medical education and teaching later.

A Warning from my Chair

The first day on the job in January 1976, I had a meeting with Ernie Knobil during which time he outlined the departmental expectations of junior faculty for research, service and teaching, the "three legs" of the academic stool. It was friendly and positive and I was eager to accept the challenge. One comment was surprising as the meeting ended. Ernie warned me that I may receive some criticism from faculty members in the school because of my use of an invertebrate model system. Ernie was aware that research on invertebrate systems was not well accepted in medical schools despite the great achievements of Eric at NYU and Columbia and John Nicholls at Stanford using invertebrate models. Fortunately, I never encountered any overt bias at Pittsburgh, and Ernie and Stan were stalwart supporters of me and my *Aplysia*. A general bias against the use of invertebrate model systems persists in some quarters, as do biases against particular invertebrate model systems.

My First Grant

Ernie "encouraged" incoming faculty to submit grant applications before actually arriving in the department. (A departure from today's expectations for new faculty who work for a year or more supported by their start-up funds to generate preliminary data for a proposal.) While at Columbia, I submitted a grant proposal to the National Institute of Mental Health (NIMH) to study the neuronal control of behavior. The ambitious plan was to analyze quantitatively the membrane currents of the ink motor neurons and to analyze and model all of the components of the gill-withdrawal reflex. The proposal received a good score but just missed the NIMH payline. A big break came when my proposal was noticed by Karl Frank, head of the Division of Grants and Contracts at National Institute of Neurological and Communicative Diseases and Stroke (NINCDS) and he agreed to "pick it up." I am indebted to him for the lift that allowed me to jump start my research with R01 NS01311 "Analysis of the Neuronal Control of Behavior." That grant included support for a technician and a state-of-the-art DEC PDP-11. The word got out that I had a new computer, prompting visits from

other faculty members within and outside the department. I was the coolest guy in town even though I worked on a snail. As I write this autobiography in 2023, the PDP-11 and its 16K of memory are long gone, but I am pleased to say that the grant has been competitively renewed ever since then. I am deeply grateful to the NINDS for their enormous continuing support.

Becoming a Member of the Medical School "Academy of Educators"

Like most faculty in basic science departments in medical schools, I came to education from a background in research. Nevertheless, I turned out to be a good teacher of neuroscience, due in part to coaching from my mentors and observation of how they teach. This is the tradition of "on-the-job training" that goes back to the Middle Ages, if not earlier. Leonardo Da Vinci, famously said, "Poor is the pupil who does not surpass his or her master." So, the bar can be set pretty high.

The Importance of Mentors

I was fortunate to have three fantastic mentors; Eric Kandel, Ernie Knobil, and Stan Schultz. Eric realized early on that progress in understanding the brain was going to require a multidisciplinary approach, and he therefore assembled a group of scientists that included psychologists, electrophysiologists, anatomists, and biochemists. It was an exciting environment, but a real challenge to the lab members, because to communicate with other lab members, you needed to be able to describe your results and ideas at a level they could understand. We were all students and teachers at the same time, and we honed our skills during our daily interactions, including lab meetings and journal clubs. From those presentations, I also discovered one of the great rewards of teaching. It's the personal gratification that you receive from successfully explaining difficult concepts to a group of "students."

Eric was an inspiring teacher. I had an opportunity to attend the introductory lecture that he gave in the newly established neuroscience course at NYU. He talked about the Greek philosophers and their attempts to understand the mind, Descartes, art, love, hatred, and how if you really want to understand the human condition and yourself, not to mention brain disorders, you need to understand the basic principles of the nervous system. At the end, there was an enormous applause from the class. I had some inspiring teachers in high school and college, but Eric was at another level. Miles Monroe, the character played by Woody Allen in the movie *Sleeper* who famously said that the brain is his second favorite organ, would certainly change his mind if he had heard Kandel's lecture that day. Eric inspired students to learn about the brain, and he inspired future teachers to inspire their students.

Practice Is the Key to Good Communication

During my stay in the lab, I also learned one of Eric's keys to successful communication. It was practice. Often before he would leave town to present a lecture at another university, he would present the lecture to the lab group for feedback. And sometimes he came back a second time after he had worked on it, based on the suggestions that he had received. So here was a member of the National Academy of Sciences and a future Nobel Laureate who practices lectures to his lab group. I learned that no matter how good you are, or think you are, you can probably be even better with some practice.

Then came my experience as a junior faculty member in the Department of Physiology at the University of Pittsburgh. Giving a good journal club presentation to a group of scientific colleagues was one thing, but was that scalable to giving a lecture to 150 first-year medical students? At the time, the sole responsibility of the department was to teach the course in mammalian physiology to first-year medical students. Ernie's philosophy was crystal clear. Excellence in teaching was expected, and it was a privilege to teach in his physiology course. The department had ownership of the course, and the faculty took great pride in it. It had the tradition of always being rated by the students as the best basic science course and was known as the course whose faculty routinely received Golden Apple teaching awards. But it was not just student feedback that was solicited, which can be fickle depending on the difficulty of the exams. Every lecture was attended by Ernie and the faculty of the course, who sat in the back row and gave feedback to the lecturers, especially the new ones. The other junior faculty warned me about the intense scrutiny and that I should expect to be summoned to Ernie's office after my first lecture. I do not think the fear factor was helpful, but it was clear that the expectations were high. But that was okay with me. I had more confidence in myself than did the other assistant professors.

That was until I found that my first teaching assignment was to give the lecture on temperature regulation; a topic that I knew virtually nothing about. (I soon learned that assignment was considered a rite of passage for new faculty.) To do it right, you needed to know multiple systems: the cardiovascular system and blood flow through the skin and muscle, sweat glands, cold and hot receptors, hypothalamic control systems, motor control of shivering, and endocrine control of cellular metabolism. To be clinically relevant, one should include hypothermia and fever. So, I had some learning to do before I could teach anything. I actually came to know and love temperature regulation. It is a great example of homeostasis and the integration of multiple systems and regulatory processes. Following the lead of Eric, I practiced that lecture—and practiced—and practiced—and practiced. To this day, my wife Susan who served as my surrogate audience, recalls the agony I put her through. Two good things did come out of those practice sessions, however. First, I got some good feedback from Susan on how to improve the lecture. Second, Susan knows more about temperature regulation than most medical students.

I gave that lecture and it seemed to be well received by the students, but my confidence was temporarily shattered when I received the dreaded call summoning me to Ernie's office. Well, Ernie liked the lecture; he just had some constructive comments for the next time. And, before long, I was asked to do additional lectures for the next year's course. This was not a burden, but an honor. I passed the test. I was now more than just a scientist in an academic department—I was a member of the "academy of educators."

Swimming Is an Attempt to Stay Alive in Water

During my time at Pittsburgh, there was a lot of talk among the faculty about pedagogy, a term I had not encountered much in the past. Pedagogy was a hot topic, because we all had to lead small-group conferences on various topics in the physiology course, ranging from electrical signaling in nerve cells to the countercurrent multiplier system in the kidney. We were all trying to outdo each other to find ways to explain difficult topics, not just to the students but to each other. As in Kandel's lab, we were again teachers and students at the same time. Of all the great teachers in the department Stan Schultz was clearly the master of pedagogy and a mentor of us all. Stan had the unique ability to explain difficult concepts to the students by using examples and humor. I remember his vivid explanations about how a pair of charged molecules move sequentially *at the same speed* through a membrane channel by saying it was just like a poor swimmer like him being tethered with a large rubber band to an Olympic swimmer like Mark Spitz. To emphasize that he was a poor swimmer Stan would say "To me, swimming is an attempt to stay alive in water." So, you could envision the two "swimmers," the graceful Spitz followed by the floundering Schultz moving through that channel together at the same speed, but with Spitz always in front! If Stan could make molecules moving through a channel interesting, I felt that I had a chance at making the brain interesting. I always think about Stan when I struggle to explain a difficult concept.

So, the extent to which I have been successful in education comes from the tremendous mentoring that I had during the early years of my career. The one problem with having Kandel, Knobil, and Schultz as mentors is the difficulty in getting a passing grade using Leonardo's criterion, but I will keep trying. I agree with Ernie Knobil that it is a privilege to be able to teach our students. It is also a privilege to be able to inform the public about the research we do. I will discuss my efforts in scientific outreach later.

Steps to Becoming a Chair

In 1980, a strong group of sensory neuroscientists in the Department of Pharmacology was transferred to the Department of Physiology and a section on neuroscience was established in the department. A national

search was initiated for a vice chair to recruit additional faculty. In the end, Ernie asked me to take the position. I saw it as a great opportunity. I became the director of the medical neuroscience course and filled four new slots. We hired Terry Crow, who worked on mechanisms of associative learning in the marine mollusk *Hermissenda*; Dan Simons, who worked on the rodent barrel cortex; Joe Yip, who worked on the development of chick spinal ganglia; and Ken Larsen, who worked on motor cortical circuitry. We were off to a great start, although things were about to change. But first, back to the research.

Biophysics and Behavior

The goal of my first NIH grant was to take a deep dive into the neuronal circuit for the gill-withdrawal reflex and quantitatively analyze the properties of the ink motor neurons. This project actually started when I was a postdoc collaborating with John Koester, who had been a postdoc with Eric, but who now was an assistant professor. We had become good friends during our time at NYU, when John worked on the neuronal control of the heart. During that time, we began a collaboration to study inking behavior and search for neurons that mediated spontaneous respiratory pumping, a fixed action pattern involving an all-or-nothing contraction of the gill and siphon, as well as inhibition of the heart.

Intracellular recordings from molluscan neurons in the 1960s and 1970s revealed a striking diversity in the electrophysiological properties of molluscan neurons, unlike the squid giant axon, which had become the prototypic neuron for all species. The giant axon was normally silent and then increased its firing activity proportional to the intensity of a stimulus. In contrast, some molluscan neurons showed dramatic adaptation of firing rate with a prolonged stimulus, whereas others showed delayed firing in response to a stimulus. Some neurons, such as *Aplysia* neuron R15, had endogenous bursting activity—cyclical bursts of spikes in the absence of a stimulus. The findings were intriguing, but we did not know if these different firing patterns had any behavioral significance. *Aplysia* offered a system to address the issue.

The L14 Ink Motor Neurons

Tom Carew and Eric Kandel discovered three nearly identical neurons designated L14, which were motor neurons controlling the inking reflex. Unlike the siphon-elicited gill withdrawal reflex, which is graded as a function of stimulus strength, Tom and Eric had found that the inking reflex has a high threshold and was elicited in a relatively all-or-nothing fashion. Moreover, they found that this high behavioral threshold was due to the high firing threshold of the L14 neurons, which they suggested was due to

the combination of an unusually high (negative) resting potential and the activation of a voltage-dependent early outward current.

While at Columbia, I began a collaboration with John Koester and two of his postdocs, Norbert Dieringer and Eli Shapiro, to examine the inking reflex and its underlying biophysical mechanisms. We focused on the question of how behavioral responses vary as a function of stimulus duration. For example, are behavioral responses that are smoothly graded as a function of stimulus intensity also smoothly graded as a function of duration; and are behaviors that are steeply graded functions of intensity also steeply graded as a function of duration? The inking and gill withdrawal reflexes provided great systems to address these questions. We found that ink release was selectively responsive to longer duration stimuli and increased in a steeply graded fashion as duration increased. This behavioral feature was associated with a distinct delay in firing followed by an accelerating spike train in the L14 neurons during a prolonged depolarizing stimulus. In contrast, the time course of the gill-withdrawal reflex was a graded function of stimulus duration. I designed and built a voltage-clamp amplifier to analyze the underlying biophysical mechanisms of the L14 responses.

In parallel experiments at Columbia and at Pittsburgh, we first characterized a depolarization-activated fast-transient K^+ current ($I\text{-}K_{V\text{-}A}$) in L14 cells. We found that the high resting potential (-75 mV) of L14 cells ensures that the steady-state level of inactivation of the $I\text{-}K_{V\text{-}A}$ conductance channels will be low at rest. Thus, a train of EPSPs can activate this current maximally, reducing the initial effectiveness of the excitatory input. Therefore, cumulative inactivation of $I\text{-}K_{V\text{-}A}$ by a prolonged train of EPSPs is required before the input can initiate a spike train. The results suggest that a unique combination of biophysical properties of the L14 cells and the features of the synaptic input cause them to act as a low-pass filter in the reflex pathway for inking. Their high resting potential makes these cells preferentially responsive to strong stimuli, and in combination with the $I\text{-}K_{V\text{-}A}$ channels, to long-lasting stimuli. The delayed recruitment of a decreased conductance EPSP augments the low-pass nature of the L14 cells. We published a paper describing this work in the *Journal of Neurophysiology* in 1979.

The next step was to prove that the properties of the $I\text{-}K_{V\text{-}A}$ and decreased conductance PSPs determined the firing properties of the L14 neurons and the behavior. To address this issue, I hoped to perform an analysis parallel to what Hodgkin and Huxley did for the ionic mechanisms of the action potential of the squid giant axon. The first task, then, was to understand those classic papers. I had a superficial understanding, but needed to go much deeper. I spent weekends in Pittsburgh reading and rereading those papers until I was ready to perform a detailed voltage-clamp analysis of all the currents. I developed a conductance-based mathematical model, and, using the model, showed that the A current was indeed sufficient to account for the features of the cell firing and the behavior. This study was not only a

significant technical achievement, but also it was the first time anyone had built a quantitative biophysical conductance-based model of a neuron that accounted for, and predicted, features of a behavior.

I thought it was a tour de force and submitted back-to-back papers to the *Journal of Physiology*, the one that published the classic Hodgkin and Huxley series in 1952. However, my bubble was soon burst when the papers were rejected, and I felt dejected. With great apprehension, I told Ernie Knobil about my failure. He was extremely supportive and encouraged me "not to take it too hard." It was a good lesson to learn, because it prepared me for future rejections and the realization that sometimes there are disagreements about what is significant and what is not. I remember John Garcia saying that his now-classic paper on food-avoidance conditioning was rejected multiple times before it was accepted for publication. Lesson learned: You just need to move on, and that is what I did. Revised versions of the papers were soon accepted in the *Journal of Neurophysiology*.

Search for Interneurons That Mediate Spontaneous Respiratory Pumping: A Fixed Action Pattern Involving an All-or-Nothing Contraction of the Gill

Tactile stimulation of the siphon elicits a withdrawal of the gill and siphon, but that withdrawal, as first noted by Kupfermann and Kandel, has two components; a rapid initial graded response followed later by an all-or-nothing component. The early classic studies of habituation and dishabituation of the reflex focused on the early component and the role of the sensory neuron-to-motor neuron connection in mediating it. The late component was thought to be mediated by an unidentified neuron called Interneuron II. Interneuron II activity also occurred spontaneously and mediated the coordinated withdrawal of gill, siphon, and mantle shelf; closing of parapodia; and inhibition of heart rate accompanied by a decrease in vasomotor tone.

John Koester and I independently found that the synaptic potentials previously attributed to Interneuron II are actually produced by at least three respiratory command cells. We combined our results in a joint-authored paper in 1978. I later found that one of these neurons was one of a cluster of mutually excitatory cells (the L25 cells), which were necessary and sufficient to produce the activity of other interneurons and motor neurons activated during spontaneous respiratory pumping. The L25 neurons were also necessary and sufficient for the all-or-nothing component of the activation of gill motor neurons elicited by siphon stimulation. Discovering the L25 neurons was special for me. We had demystified the elusive Interneuron II. The finding was not earth shattering in the broader neuroscience community, but, for me, it was a homerun.

The Interneuron II story is not over, however, and the extent to which plasticity of the L25 neurons contributes to habituation, dishabituation, and sensitization of the gill-withdraw reflex has not been examined. Perhaps the

degree of their engagement simply follows the decrement and enhancement of the sensory neuron connection that occurs at motor neurons. Perhaps the plasticity rules are different. There could still be an additional and major unexplored dimension to understanding the gill-withdrawal reflex and its modification by learning.

My First Book

This growing interest in the unique biophysical properties of neurons led to a 1980 Banbury Conference at Cold Spring Harbor Laboratory (CSHL) organized by Eric Kandel and Chuck Stevens. Chuck and Eric asked John Koester and me to edit a volume of the proceedings to be published by CSHL Press. *Molluscan Nerve Cells: From Biophysics to Behavior* was my first book, and I was excited for the opportunity to summarize the ways in which the unique membrane currents of neurons contribute to their information processing, an issue that has become of great interest and importance in the vertebrate CNS. It was also the start of a long relationship with CSHL and CSHL Press. Over the years, I have had the privilege of organizing many meetings and courses and of serving as editor-in-chief of *Learning and Memory* between 1996 and 2024.

Back to Synaptic Plasticity and Finding the Holy Grail for Associative Learning

Synaptic Plasticity and a Quantitative Model of Transmitter Release and Its Plasticity

A goal of my first NIH grant was to analyze quantitatively the properties of the sensory neurons in sufficient detail to construct a model of the release process. This model would be a component of the complete withdrawal circuit and the plasticity that underlies habituation and sensitization. Marc Klein and Eric Kandel had suggested that the decrement of transmitter release associated with habituation was due to cumulative inactivation of Ca^{2+} current in the sensory neurons. Sensitization was believed to be caused by a serotonin (5-HT)- and cAMP-mediated reduction of a depolarization-activated membrane current, the S-K^+ current. Reduction of the S-current would lead to broadening of the action potential, more calcium influx and greater transmitter release (i.e., synaptic facilitation). The model of cumulative inactivation would predict that synaptic depression would be a monotonic function of stimulus interval. Other single-process assumptions such as simple depletion of transmitter would make similar predictions. Instead, I found that synaptic depression varies as a complex function of interstimulus interval and that the recovery time course of synaptic depression following a train of stimuli is variable. Shorter spike intervals produced

more rapid recovery. I suggested that intracellular accumulation of Ca^{2+} at short spike intervals contributed to the rapid recovery. Clearly, things were more complex than would be predicted by a single process, and a new model was needed.

Around this time, I was fortunate to be approached by Kevin Gingrich, a first-year medical student with an undergraduate degree in electrical engineering from Cornell University who was looking for a project that involved modeling. I had just the project for him! We developed a lumped parameter model of the sensory neuron release process, which included Ca^{2+}- channel inactivation, Ca^{2+}-mediated neurotransmitter release and mobilization, and readily releasable and upstream feeding pools of neurotransmitter. The model not only simulated the data of synaptic depression and recovery from depression, but also qualitatively predicted other features of neurotransmitter release that it was not designed to fit. The model could not account fully for synaptic depression with only the empirically observed somatic Ca^{2+}-current kinetics. Rather, the model predicted that a large component of synaptic depression was due to depletion of the pool of releasable transmitter. Kevin found time to continue the project during his four years of medical school and as a resident, when we expanded the model to include processes for associative learning.

Associative Learning and the Battle Between the Crunchies and the Squishies

In the late 1970s and early 1980s, the Kandel and Schwartz labs were making great strides in elucidating the role of cAMP in mediating the presynaptic facilitation associated with sensitization, an example of nonassociative learning. But despite the progress, some in the field were not impressed because sensitization was thought to have little relevance to vertebrate learning. The real "brass ring" was to understand mechanisms of associative learning, such as classical (Pavlovian) conditioning, and operant (instrumental) conditioning, and it looked like *Aplysia* was lagging behind some of the other invertebrate model systems in this aspect. Teresa Audesirk at the University of Missouri demonstrated associative conditioning of feeding behavior in *Lymnaea*; Jack Davis and his group at the University of California, Santa Cruz, demonstrated classical conditioning of feeding behavior in *Pleurobranchaea*; Alan Gelperin at Princeton demonstrated food aversion learning in *Limax*; and Terry Crow and Dan Alkon at the NIH demonstrated classical conditioning of *Hermissenda*, along with neuronal correlates of the behavioral changes. There were also examples of associative learning in arthropods, such as honeybees, *Drosophila*, and cockroaches. Graham Hoyle said during an SfN talk that "it was a battle between the crunchies and the squishies" for who would get that brass ring. The 1981 finding by Tom Carew, Terry Walters, and Eric Kandel that the

siphon-elicited siphon-withdrawal reflex could undergo classical conditioning was the major advance that allowed *Aplysia* to catch up. Now the big challenge was to discover the underlying mechanism.

At that time, I was fortunate to have Terry Walters join the lab as a postdoctoral fellow. We began thinking of possible mechanisms for classical conditioning. At the most fundamental level, and neglecting the constraints on temporal order, classical conditioning is an *and* gate phenomenon. The conditioned response (CR) comes about after the conditioned stimulus (CS) *and* the unconditioned stimulus (US) are paired. Presentation of the CS alone or the US alone did not lead to conditioning. One possibility was that the mechanism of associative learning was an elaboration of mechanisms already in place that mediate sensitization. For example, the aversive US likely caused the release of 5-HT and subsequent activation of the cAMP cascade (i.e., one arm of the AND gate), but what was the CS pathway? Here we were influenced by a growing body of literature showing interactions among second-messenger cascades, in particular calcium and cAMP, as elegantly summarized in Howard Rasmussen's 1981 book *Calcium and cAMP as Synarchic Messengers*. There was good evidence to suggest that the burst of spikes activated by the CS in sensory neurons would lead to an influx of calcium, which in turn could modify the adenylyl cyclase complex and potentiate cAMP levels beyond those elicited by the US alone. We tested the hypothesis using an *in vitro* analog of classical conditioning consisting of artificial activation of sensory neurons (CS) in the pleural ganglion paired with shock to the tail (US). We found that sensory neurons activated shortly before a sensitizing shock displayed significantly more facilitation of their monosynaptic connections to tail motor neurons than cells trained with control treatment. This associative effect was acquired rapidly and was expressed as a temporally specific amplification of heterosynaptic facilitation. We termed this mechanism activity-dependent neuromodulation.

During the course of our experiments, we learned that Tom Abrams, Tom Carew, Bob Hawkins, and Eric Kandel were pursuing similar experiments and were obtaining similar results using the sensory-to-motor neuron connections in the abdominal ganglion. We were ready to submit our paper, but Eric suggested that we hold back and allow them to finish up at which time we would submit back-to-back papers to *Science*. The idea was that two papers showing the same basic effect in two different reflex systems would have a greater impact than either one alone. Albeit tongue-in-cheek, Eric was fond of saying, "If one group discovers a mechanism in *Aplysia* it is general, but if two groups discover the same mechanism, it is universal." We agreed to Eric's suggestion and the papers received great reviews. One of the reviewers, who later revealed himself to be William "Chip" Quinn at MIT, said in his review that we had found the holy grail of learning.

The University of Texas Health Science Center at Houston

The University of Pittsburgh Medical Center is now one of the premier centers in the world for basic and clinical research. But in the late 1970s and early 1980s, the ship was at the point of foundering. There were cuts in the state budgets and serious problems with the organization of the various affiliated hospitals. Some good people started to explore other opportunities. One great personal and professional loss was the recruitment in 1979 of my colleague and friend Stan Shultz to chair the Department of Physiology at the University of Texas (UT) Medical School at Houston. Admittedly, it was a great opportunity for Stan, but a loss for Pittsburgh. The Texas medical school was less than 10 years old and aspired for greatness. Stan's charge was to take the Department of Physiology to the next level by recruiting an outstanding faculty. I was flattered at a going away dinner for Stan, when Ernie wished him great success, but admonished him to "stay away from recruiting Jack Byrne." Stan started to build the department and also quickly became an academic leader in the school. One of his new jobs was to serve as the chair for the search committee for the dean of the school. And you might have anticipated who would be the prime candidate. At a faculty meeting in 1981, Ernie dropped the bombshell that he was leaving Pitt to become dean of the UT Medical School at Houston. Ernie's goal was to make UT Houston one of the top 10 public medical schools in the country. I felt a bit abandoned with Ernie and Stan's departures. Leonard "Rusty" Johnson, a visiting speaker from Houston "rubbed it in" by beginning his seminar saying, "It's good to see some people are still left in Pittsburgh." I started to look for a new boat, and naturally Houston seemed like a good option. So, in July of 1982, I started my new lab in the newly named Department of Physiology and Cell Biology. Some 14 years earlier, I had said I would never move to Houston. Yet, here I was with a golden opportunity.

Houston also provided an opportunity to get back to the sea. I started racing sailboats on Galveston Bay and the Gulf of Mexico, first in a crew on various J boats (J/29, J/32, J/35, and J109) and eventually with my own J/22 and later J/80. Now my daughter, Kathryn, and son, Michael, were the crew members. I was not the only sailor/scientist at the Houston Yacht Club. Tom Caskey, pioneering geneticist from Baylor; and our neurologist UT Houston President M. David Low were active sailors. Soon Ernie Knobil got the bug and bought a 37-foot Westerly.

Boom Times in the 1980s and 1990s

The "oil boom" in Houston went bust soon after I arrived, but my research started to really flourish. This scientific boom was due to several outstanding colleagues who joined the lab. With graduate students Dean Buonomano, Jennifer Raymond, Ken Scholz, and John Walsh; postdoctoral fellows Len

Cleary and Karen Occor; senior research associate Doug Baxter; and visiting professor Avy Susswein, I continued several lines of research started in Pittsburgh and embarked on new areas, such as the neural circuit for the tail-elicited tail withdrawal reflex, the mechanisms for long-term sensitization (LTS), nonlinear dynamics of neurons and neuronal networks, and the neuronal control of feeding behavior and its plasticity.

Back to Mechanisms of Classical Conditioning

After the back-to-back *Science* papers with Eric's group, the hunt was on in both groups for the biochemical mechanism of activity-dependent neuromodulation. Both papers suggested that the mechanism for activity-dependent neuromodulation was the activity-induced influx of Ca^{2+} interacting with the serotonin-sensitive adenylyl-cyclase to potentiate cAMP levels, but we needed the evidence.

In prepandemic times, a successful career in science led to invitations to present your results to colleagues at other universities. A great benefit of these visits is meeting with students, postdocs, and faculty and learning about ongoing research, which in many cases can help inform and direct your own research. In addition, some of the students and postdocs with whom you meet are looking for future opportunities. A visit to Wesleyan University in 1981 turned out to be particularly fortuitous, because it was there that I met Karen Occor. She was finishing up her doctoral project under the supervision of Allan Berlind, working on the identification and localization of a catecholamine in the motor neurons of the lobster cardiac ganglion. She soon thereafter joined my lab as a postdoc.

Karen developed an *in vitro* analog of classical conditioning, consisting of isolated clusters of sensory neurons and the use of a brief application of a high K^+ solution to mimic the CS and a brief application of 5-HT to mimic the US. Karen found a pairing-specific amplification of the cAMP content of isolated sensory neurons. The enhancement was achieved within a single trial. These results indicated that a pairing-specific enhancement of cAMP levels may be a biochemical mechanism for associative neuronal modifications. Tom Abrams and his colleagues pursued the question further and in an elegant series of experiments examined the temporal order of the pairing effects.

The Serotonin-Induced Modulation of Membrane Currents Was More Complicated Than We Thought

According to the early model of Kandel and colleagues, sensitization was believed to be caused by a serotonin (5-HT) and cAMP-mediated reduction of a specific membrane current, the S-K^+ current. Reduction of the S-current would lead to broadening of the action potential, more calcium influx, and greater transmitter release (i.e., synaptic facilitation).

Doug Baxter wrote to me in 1984 about his desire to work on synaptic plasticity in *Aplysia*. It was an easy decision to bring him on board. He had already done excellent work on synaptic plasticity of the crayfish neuromuscular junction during his doctorate with George Bittner and postdoc with Tom Brown. Moreover, I had the necessary resources having just received new funding from the Air Force Office of Scientific Research (AFOSR). Doug turned out to be fantastic in every way; he was extremely smart and technically competent. And, he was one of the nicest and most generous scientists whom I have met. I have been extremely fortunate that our fruitful collaboration has persisted for almost 40 years. In 1985, Doug set out on an ambitious task to perform a complete voltage-clamp analysis of the membrane currents of the sensory neurons and their modulation by 5-HT. The project built on the earlier work of graduate student John Walsh, who found that 5-HT modulates a steady-state calcium-activated K^+ current. Doug extended the analysis by examining the voltage-activated $I_{K,Ca}$ and the delayed outward current. He found that that 5-HT modulates not only the S- K^+ current but also the delayed K^+ current and a voltage and Ca^{2+}- activated K^+ current. Subsequent studies showed that the S-K^+ current was important for changes in excitability. In contrast, the delayed K^+ current contributed importantly to the increased duration of the action potential. Moreover, through later work by graduate student Shuzo Sugita, we found that the effects of 5-HT on the delayed K^+ current were predominantly modulated by protein kinase C (PKC). An important conclusion from these studies is that sensitizing stimuli affect a host of processes in the sensory neurons, all of which act synergistically in a state- and time-dependent manner to enhance the release of transmitter and bring about an enhanced withdrawal response (i.e., sensitization). This engagement of multiple messenger systems and substrates elucidated in *Aplysia* has emerged as a general principle of synaptic plasticity. Eric and I had an opportunity to review mechanisms for short-term sensitization in our 1996 *Journal of Neuroscience* paper "Presynaptic Facilitation Revisited: State- and Time-Dependence." Things have gotten a bit more complicated since then, with the involvement of additional autocrine and postsynaptic processes contributing to short-term facilitation, which Bob Hawkins and I reviewed in our 2015 paper in *Cold Spring Harbor Perspectives in Biology.*

Neural Circuit for the Tail-Elicited Tail Withdrawal Reflex

Len Cleary, who completed his doctorate with Jimmy Schwartz at Columbia in 1984, wrote in a letter to me that he was "most interested in starting with a physiologically interesting cell and analyzing its connections morphologically." I invited Len to join the lab and have had the pleasure of working with him ever since. He made the outstanding discovery of a multifunctional interneuron LPl17 that was critical for mediating the tail stimulus-elicited

tail withdrawal and linking sensory information from the pleural ganglion to the abdominal ganglion. That link led to activation of gill and siphon motor neurons and the Interneuron II (L25) network. It was more than a link, however. It transformed the short-lasting synaptic input it received to a slow and long-lasting response in the postsynaptic neurons. It is not generally appreciated that measures of sensitization frequently use *duration* as an index of the degree of behavioral change, and these changes in the duration can last many seconds. However, mechanisms of sensitization of defensive reflexes have been correlated primarily with changes in the *amplitude* of the monosynaptic EPSP. Neurons like LPl17 allow the changes in PSP amplitude to be transformed to changes in duration. A complementary study by postdoctoral fellow John White used a three-layer circuit model to demonstrate the sufficiency of LPl17 to mediate this transformation.

The 1985 Dahlem Conference and the Mechanisms of Long-Term Memory

My early work focused on the analyses of short-term behavioral and synaptic changes underlying habituation, sensitization, and classical conditioning, but my interest in long-term mechanisms was stimulated during a 1985 Dahlem Conference in Berlin organized by J.-P. Changeux and Mark Konishi. It was a Who's Who of learning and memory scientists including, to name a few, Per Andersen, Mark Bear, Michel Baudry, Tim Bliss, Yadin Dudai, Hersch Gerschenfeld, Masao Ito, Mary Kennedy, Randolf Menzel, Michael Merzenich, Mort Mishkin, Roger Nicoll, Pasko Rakic, Edmund Rolls, Wolf Singer, and Richard Thompson. Invertebrates were well represented with me, Tom Carew, and Eric Kandel (*Aplysia*); Dan Alkon (*Hermissenda*); Randolf Menzel (*Apis*); and Yadin Dudai and Martin Heisenberg (*Drosophila*).

It was a wonderful conference and over those four days and nights of scientific discussions and socializing, I developed lasting friendships with many of the participants. We were divided into four discussion groups: (1) activity-dependent regulation of gene expression; (2) activity-dependent regulation of synaptic transmission and its relationship to learning; (3) activity-dependent modification of functional circuitry as a possible basis for learning; and (4) neuronal assemblies and memories. I was assigned to the group on activity-dependent regulation of synaptic transmission and its relationship to learning. We had some great discussions that were summarized in a subsequent book chapter by our rapporteur Michel Baudry. One of the great features of the format for the Dahlem conferences was the staggering of the sessions so that members of one group could "visit" other groups. I was particularly impressed with the excitement of the members and importance of the question being considered by the subgroup on activity-dependent regulation of gene expression. The idea that protein synthesis was important for long-term memory was becoming well established, but little was known about the identity of individual proteins and how they were

regulated. I decided then that the tail-elicited tail and siphon-withdrawal reflex might have something to contribute.

Ken Scholz, a new graduate student who did his undergraduate work in mechanical engineering at Boston University, showed that the reflex had a memory for sensitization that persisted for at least a day, and, importantly, that LTS training led to a reduction in net outward current in the sensory neurons. The results indicated that one mechanism for the storage of LTS is the regulation of membrane currents that influence the characteristics of the action potential and the excitability of individual neurons. The results also provided insight into the relation between short-term sensitization and LTS in that the biophysical loci involved in the storage of LTS appeared to be similar to those involved in short-term sensitization. But what about the induction? Ken demonstrated that cAMP was as critical for the induction of LTS as for short-term sensitization. Injection of cAMP into individual neurons in isolated ganglia led to a reduction of outward currents similar to that produced by behavioral training. The current that is reduced over a long period of time (24 h) as a consequence of cAMP injection and behavioral training had relatively slow kinetics and mild voltage dependence and was active close to the resting potential. This result indicates that long-term reduction of this current was involved in enhancing the excitability of the sensory neurons and in the control of repetitive firing. The Kandel group was actively pursuing the role of cAMP in LTS as well. Sam Schacher, Vince Castellucci, and Eric found that bath application of a cAMP analog led to a long-term (24 h) enhancement of the EPSP in sensorimotor cocultures. Our complementary findings led to another set of back-to-back papers in *Science* in 1988. cAMP was doing more than inducing changes in membrane currents, however. Graduate student Fidelma O'Leary found that cAMP also produced morphological changes in the sensory neurons, including increased numbers of sensory neuron branches and varicosities. These changes required *de novo* protein synthesis. Thus, there is not a single expression mechanism for LTS. It is expressed both by modulation of membrane currents and by morphological changes in sensory neurons.

A constant concern of using reduced preparations such as isolated ganglia or neurons in culture (or vertebrate slice preparations) is the extent to which phenomena at reduced levels map onto changes *in vivo*. My strategy was to try to validate findings at these different levels. Graduate student Marcy Wainwright, working with Len Cleary, showed that LTS was associated with morphological changes in the sensory neurons, including an increased number of sensory neuron branches and varicosities. And postdoc Wai Lee, working with Len Cleary, showed in 1998 that LTS was associated with changes in synaptic strength and changes in excitability of sensory neurons. Interestingly, they also showed for the first time that the biophysical properties of the postsynaptic motor neurons were affected by LTS. An independent study by Louis-Eric Trudeau and Vince Castellucci in Montreal

showed that the response in a gill motor neuron in an isolated abdominal ganglion to a glutamate agonist was enhanced 24 hours after a 60-minute treatment with 5-HT. Subsequent studies by the labs of David Glanzman at University of California, Los Angeles (UCLA) and Bob Hawkins at Columbia have greatly expanded the understanding of postsynaptic induction and expression mechanisms of LTS.

Feeding Behavior in Aplysia and Its Modification by Classical Conditioning

Although my lab had made great progress in analyzing mechanisms of classical conditioning at the sensorimotor synapse, that model system is not ideal. For example, there is a large nonassociative component to both the behavior and the synaptic facilitation, and the measure of the conditioning, the amplitude of the EPSP, is somewhat removed from the common measure of the behavior, the duration of siphon withdrawal. The duration of siphon withdrawal is difficult to measure, and the conditioned stimulus (touch to the skin) is difficult to quantify and deliver. Moreover, the conditioning used an aversive training procedure (i.e., shock). For comparison with vertebrate systems, it is desirable to use an appetitive conditioning protocol. Consequently, we were drawn to the feeding behavior in *Aplysia.* Avy Susswein had discovered that animals were capable of learning that food was inedible if wrapped in a plastic net, a form of associative conditioning.

A major challenge was the lack of understanding of the circuitry that controlled feeding behavior. It was not that nothing was known. Indeed, Dan Gardner, a graduate student of Eric in the early 1970s, had identified many of the neurons in the buccal ganglion and their synaptic connections. Irv Kupfermann had made major strides working with Klaude Weiss on identifying motor neurons and modulatory transmitters controlling the buccal mass. They had also identified neurons that controlled the motivational state of the animal. The major gap was the identity of neurons that *initiate* the buccal motor program for feeding.

Avy Susswein and I met as graduate students at NYU. Avy was with Irv Kupfermann, who had recently set up his own lab to use feeding behavior as a model system to study motivation. Avy's thesis examined satiation of feeding behavior, which he continued when he secured a faculty position at Bar Ilan University. There he made the discovery in 1983 of associative learning of feeding behavior. Avy was a terrific behaviorist, but he lacked experience in cellular neurobiology, so he had difficulty pursuing the mechanisms underlying that form of learning. He approached me in 1985 to do a sabbatical in Houston to get that expertise. It was a great partnership. Avy brought the feeding system to my lab, and he learned intracellular recording techniques. Although we did not uncover the mechanism of associative learning, we did discover a key neuron (B31) that was necessary and sufficient

for initiating the buccal motor program. That finding laid the ground work for subsequent work in my lab with Doug Baxter, as well as Avy's lab and that of Hillel Chiel, Betsy Cropper, Mark Kirk, Irv Kupfermann, and Klaude Weiss, expanding details of the circuitry mediating buccal motor programs. That collective knowledge paved the way for examining cellular correlates of associative learning.

Avy's associative learning paradigm was not ideal for pursuing a cellular analysis. We sought a simpler system in which a CS and a US could be paired in a traditional classical conditioning protocol. Two important developments occurred almost simultaneously. First, in 1997, Ruth Colwill at Brown showed appetitive classical conditioning of feeding behavior when a neutral chemical stimulus (CS) was repeatedly paired with food reinforcement (US). Second, Hilde Lechner joined my lab as a graduate student after doing undergraduate studies in Randolf Menzel's lab at the Free University of Berlin. Hilde was an exceptionally gifted student who had an excellent background in learning theory. Having worked on the tiny bee brain, she was unintimidated about working with *Aplysia*. She was the perfect person to try classical conditioning in Houston. Hilde's key insight was to use a mechanical stimulus rather than the chemical one used by Ruth Colwill. If conditioning was successful, the subsequent analyses would be facilitated because we knew more about the mechanoreceptor pathways than the chemoreceptors. Hilde's protocol was successful and she went on to show that appetitive classical conditioning of feeding resulted in the pairing-specific strengthening of the polysynaptic pathway between afferent fibers and the motor pattern-initiating neuron B31, the neuron that Avy Susswein had identified 10 years earlier.

Operant Conditioning

Whereas excellent progress had been made in analyzing mechanisms of classical conditioning by us and by other groups, at that time, virtually nothing was known about the neural mechanisms of the second major form of associative learning known as operant conditioning. We made enormous strides in demystifying this form of learning. The results are also important because they allow for a comparative analysis between these two major forms of associative learning. Although they are operationally distinct, psychologists have argued for decades about the mechanistic similarities. Progress was made possible by Romuald Nargeot, an outstanding postdoc who joined my lab, and by generous support from William Berry's program at the AFOSR. We were also inspired to examine operant conditioning of feeding behavior with the success in 1986 of Cook and Carew in demonstrating operant conditioning of head-waving behavior.

In 1994, I received a letter from Romuald asking if I would serve as a member of his thesis jury at the University of Bordeaux at Arcachon.

It was a great honor to be recognized internationally as a good mentor. It was also a special treat to visit the laboratory and see the rig room where Eric did his classic experiments with Ladislav Tauc in the early 1960s. More fortune came my way when Romuald joined my lab as a postdoctoral fellow. The goal was to develop an operant conditioning paradigm using the feeding system. Rather than start with the behavior, our goal was to develop an *in vitro* analog. This seemed possible for two reasons. First the isolated buccal ganglion was capable of exhibiting spontaneous motor programs. Thus, we had an operant that could in principle be conditioned. Second, we knew that one of the peripheral nerves innervating the esophagus was rich in dopamine-containing fibers that signaled the presence of food in the gut. Thus, we had a potential reinforcing pathway. The idea was to activate the esophageal nerve (i.e., reward) after each fictive ingestive pattern. This sounds good, but what parameters of stimulation do we use and how many times do we deliver the reinforcements? There was no *a priori* answer. It was strictly trial and error, which I will say more about later. And here is where Romuald's dogged persistence came into play. He did experiment after experiment, sometimes four in one day, exploring the "parameter space" until he found a protocol that worked. We had operant conditioning in a dish and published the result in 1997. Next Romuald explored neuronal correlates and found that changes in the excitability of neuron B51 played a major role in producing operant conditioning of the buccal motor programs.

I mentioned earlier the great benefits derived from invitations to present one's results to colleagues at other universities. Another great benefit is attending scientific meetings, especially the small ones like the Dahlem conferences. (Regrettably, the COVID era has diminished these opportunities.) During the Fifth International Conference of Neuroethology in 1998 I had the great pleasure of meeting Bjoern Brembs. Bjoern was a student of Martin Heisenberg working on operant conditioning of *Drosophila*, and he thought the *Aplysia* system might be useful for a more mechanistic understanding of operant conditioning. I invited him to join the lab. He, like Romuald, was extremely motivated and soon showed operant conditioning *in vivo*. He also showed that *in vivo* conditioning, as in *in vitro* conditioning, led to changes in the excitability of neuron B51. Graduate student Fred Lorenzetti took the analysis one step further by showing that similar cellular changes in B51 were produced by contingent reinforcement of B51 with dopamine in a single-cell analog of the operant procedure. These findings allowed for the detailed analysis of the cellular and molecular processes underlying operant conditioning performed by Fred, who stayed on as a postdoctoral fellow. Fred examined second-messenger pathways engaged by activity and reward and considered how they may provide a biochemical association underlying operant learning. He found that the changes in excitability were due to the synergistic interaction of PKC and PKA (protein kinase A). Interestingly, the mechanisms of activity-dependent neuromodulation

underlying this appetitive form of operant conditioning appear to be very similar to the mechanisms underlying the aversive classical conditioning of the withdrawal reflexes. In the sensory neuron, the coincidence detection involves, at least in part, a synergistic interaction between a Ca^{2+}/calmodulin-sensitive adenylyl cyclase (type I) and a serotonin-activated cAMP cascade. Although the specific isoforms of adenylyl cyclase appear to differ, adenylyl cyclase appears to serve as the molecule of convergence in both forms of learning.

Our initial focus was on neuron B51, and early on, we thought changes in its excitability could account for a large part of the behavioral modifications. Not surprisingly, things turned out to be more complicated. Romuald set up an independent lab at the University of Bordeaux, and went on to find that operant conditioning led to changes in excitability of three other neurons (B30, B63, and B65), as well as changes in the electrical coupling among them. So, at least four neurons had changed. Surprisingly, all of the changes were in intrinsic properties of neurons (i.e., excitability) or electrical coupling. Where were the changes in chemical synaptic transmission that dogma says is the basis of learning and memory? Postdoctoral fellows Yuto Momohara and Curtis Neveu set out to address that issue by making an exhaustive search for additional changes. That led to the identification of yet another neuron (B4) whose excitability was altered by operant conditioning. In addition, they found a reduction in the strength of B4's inhibitory synaptic connection to neuron B51.

Our analyses of operant conditioning revealed changes in the excitability of five neurons as well as changes in electrical and chemical synaptic transmission. But were all of these necessary for learning? To address this issue, Momohara, Neveu, and I developed and analyzed a conductance-based computational model of the central pattern generating circuit to examine the relative contributions of plasticity loci. The model indicated that the known plasticity loci showed a surprising level of synergism in mediating the behavioral changes associated with the operant conditioning. In general, this study illustrated that focus on a single locus can give a very incomplete understanding of learning, even in a relatively simple circuit. It is necessary to understand the relationship between the different loci to understand how the network is modified.

There is still much work to be done to determine the mechanisms underlying operant conditioning. Recently, in my lab, large-scale recordings using voltage-sensitive dyes (VSDs) with single-neuron resolution have begun to reveal other putative sites of plasticity. We anticipate that we will soon elucidate the complete scope of plasticity changes and the complete wiring diagram of the feeding circuit. I have great hope that the feeding system of *Aplysia* will ultimately provide a comprehensive understanding of the ways in which memories are encoded in a relatively simple circuit, elucidate design principles of memory encoding, and provide guidance for similar analyses in more complex systems.

Getting Molecular: The Search for Memory Proteins

You know by now that I was not trained as a biochemist or molecular biologist, but one of the great aspects of a career in science is the ability to learn and apply new techniques to address your research question. (Indeed, it might be said that a failure to do so jeopardizes your progress.) So, I had to get some expertise in molecular biology if I wanted to identify the genes and proteins for long-term memory. Harel Shouval, a colleague in my department, is fond of saying that "an airplane does not fly if it does not keep moving forward."

I was fortunate to begin a collaboration with Arnold Eskin at the nearby University of Houston. Arnold had done outstanding work on the molecular mechanisms of circadian rhythms in *Aplysia*, but he was looking to expand his research into learning and memory. It was a great partnership because I had the learning background and a good model system, and he had the expertise in molecular biology. We set out to identify genes and proteins involved in long-term memory. One of our big successes was the identification of an mRNA with significantly increased expression in sensory neurons following treatments with serotonin or long-term behavioral training. Moreover, the effects of serotonin and behavioral training were mimicked by treatments that elevate cAMP. This *Aplysia* mRNA had a high sequence homology with a developmentally regulated gene family that includes *Drosophila* tolloid and human bone morphogenetic protein-1 (BMP-1).

Both tolloid and BMP-1 encode metalloproteases that can activate TGFβ (transforming growth factor β)-like molecules. That finding led to a test of the hypothesis that in addition to its role in development, TGFβ might be a key signaling molecule in LTS. And indeed, in a subsequent paper in *Science*, graduate student Fan Zhang found that treatment with TGFβ1 induced long-term facilitation (LTF), but not short-term or intermediate-term facilitation, and that serotonin-induced LTF was blocked by an inhibitor of TGFβ. These results and a follow-up study by graduate student Jeannie Chen indicated that activation of TGFβ may be part of the cascade of events underlying LTS. Moreover, they indicated for the first time that an extracellular biochemical feedback loop was involved in LTF. In an elegant series of experiments, Tom Carew and his colleagues have further elaborated the role of TGFβ in LTF and showed that the adult nervous system reuses developmental growth molecules to promote synaptic changes in the mature brain.

TGFβ is not the only protein to change with learning. The collective work of my lab; the labs of Carew, Kandel, and Schwartz; and more recently the lab of Irina and Robert Calin-Jageman have identified many more. The message is that there is not a single memory gene or protein. Many genes are involved, and they act together to alter the biophysical and morphological properties of the sensorimotor connection. These genes also act according to a temporal pattern. Some genes are turned on early, some at intermediate

times, and some late (24 hours after training). I began to wonder what kind of control mechanisms were mediating these dynamics and whether a knowledge of the dynamics could help design better training protocols.

Chaos and Nonlinear Dynamics of Neurons and Neuronal Networks

I had a long-standing interest in neuronal dynamics going back to my earlier coursework in graduate school, but I had a great opportunity to take a deeper dive when I was approached in 1989 by Carmen Canavier, a graduate student working with John Clark in the department of electrical and computer engineering at nearby Rice University. Carmen had done her undergraduate work in electrical engineering at Vanderbilt University and was interested in the nonlinear dynamics of neurons. We came up with a project to develop an improved model of the firing pattern of the *Aplysia* endogenous bursting neuron R15. Carmen built a model that better fit the data, and the R15 story got more interesting when she found that with certain parameter settings the simulated neuron exhibited chaotic activity and multistability. With multistability, depending on the initial conditions, the neuron could be completely silent, exhibit continuous firing (i.e., pacemaker activity), or burst as usual. Moreover, a brief perturbation could switch the neuron from one mode to another. Here was a memory mechanism that was independent of any second messengers, kinases, or protein synthesis. During this time, we were very fortunate to receive successive grants from the Office of Naval Research's program on Nonlinear Dynamics headed by Tom McKenna. We received interesting comments from the *Aplysia* community when we presented these results at an SfN meeting. Almost every Aplysiologist at one time or another has recorded from R15, just to see in real time the singular beauty of its parabolic bursting behavior. But people would observe that sometimes the bursting was not there and then it would start, or it was there, and then it went away. Was the cell dying, did the temperature change, did the electrode move? None of the above. These changes were a manifestation of multistability. The field had a bit of an "Aha" moment.

The collaboration on neuronal dynamics with John Clark continued for 10 years. Carmen continued her work in the lab as a postdoc and was joined by a succession of outstanding Rice graduate students, including Rob Butera, Semahat Demir, and Ron Dror, examining phase-sensitivity, reduced models of R15, and introduction of R15 models into ring circuits. Inserting an R15 model into a simple four-neuron ring circuit could simulate quadrupedal gate patterns, including walking, trotting, bounding, and galloping. Lesson learned: even simple neuronal circuits can produce complex behaviors when there are nonlinearities in the component neurons. I still believe that many biologists do not fully appreciate the role and importance of nonlinear dynamics in biological processes. Certainly, teaching of this topic in the graduate curriculum is insufficient.

By the late 1990s, we had made great progress understanding nonlinear dynamics of neuronal activity and mechanisms of long-term memory. It was a good time to put these different strands together.

Dynamics of Gene and Protein Networks

If there is a consistent theme throughout this autobiography, it is the great strides made when new colleagues joined the lab and brought with them new enthusiasm, expertise, and ideas; a fresh breeze to fill the sails. Unfortunately, I have not been able to tell all of their stories and enormous contributions because of space limitations. But here, I do need to note the contributions of Paul Smolen who joined the lab as a postdoc in 1996. Paul received his doctorate in 1984 working with Joel Keizer at University of California, Berkeley, followed by postdocs with John Rinzel and Erik de Schutter. Needless to say, he arrived as an extremely well-qualified computational neuroscientist.

Our first paper with Paul Smolen examined frequency selectivity, multistability, and oscillations in genetic regulatory systems. One important aspect of that paper was to look at how, in principle, competition between activating and repressing transcription factors could provide an explanation for optimal stimulus frequencies that explain the advantage of spaced vs. massed learning trials. Paul went on with me and Doug Baxter to write a wonderfully comprehensive review on mathematical modeling of gene networks for *Neuron*. Then, partly influenced by Arnold Eskin's earlier work on circadian rhythms and his colleague Paul Hardin, then at the University of Houston, and with a grant from the AFOSR, we began modeling the gene and protein networks underlying circadian rhythms. We incorporated some of the newly discovered genes, and developed and published models with good predictive ability. Unfortunately, the project was terminated when the AFOSR decided, as one program officer told me, not to fund further work on any "creatures." It was unfortunate, but the work on circadian rhythms got us "tuned up" for going full throttle into gene and protein networks for memory.

There is much more great science to come, but at this point, I think I am getting too far ahead with my story. There were some major nonscientific career developments during the 1980s and 1990s. So, let's take a step back to 1986.

Building a Neuroscience Department and Getting Sunk by Allison and Other Misfortunes

I was doing just fine working in 1986 as a professor in Stan Schultz's Department of Physiology and Cell Biology, but an opening for the chair of the Department of Neurobiology and Anatomy became available, and I

threw my hat in the ring. It seemed like a potential opportunity to build neuroscience in the school (which was somewhat lacking), and in particular, to expand in the area of neuronal plasticity and learning and memory. I had no intent of curtailing my research program. My models were Ernie Knobil and Stan Schultz, who were great chairs and still maintained outstanding research programs. I was fortunate to be able to appoint Doug Baxter and Len Cleary as assistant professors. They handled the day-to-day lab operations, with Doug focusing on the feeding projects and Len focusing on the sensitization projects.

There were departmental faculty slots to fill and I knew from the 1985 Dahlem meeting which fields were creating the most excitement and who was active in those fields. In short time, we recruited Jocelyn Bachevalier to work on development of learning in primates, Terry Crow to work on mechanisms of learning in *Hermissenda*, Pramod Dash to work on gene regulation in memory, Dan Felleman to work on visual cortical plasticity, Mike Mauk to work on classical conditioning of the eyeblink response, Randy Nudo to work on motor cortical plasticity, and Neal Waxham to work on the role of calmodulin in synaptic plasticity. We were covering neuronal plasticity from molecules to networks and gaining prominence at the local and national levels. The department and the neuroscience graduate program soon went from one of the worst in the school to one of the best. We had multiple training grants and program project grants and million-dollar gifts from the Markey Trust and the W. M. Keck Foundation. That later gift established the W. M. Keck Center for the Neurobiology of Learning and Memory and allowed us to recruit Andy Bean, Ruth Heidelberger, Roger Janz, and James Knierim. In conjunction with the formation of a joint biomedical engineering department between UT Austin and Houston funded by the Whittaker Foundation, we also recruited Yin Liu and Harel Shouval. We had a fantastic group of neuroscientists, and we all shared a common goal. We were on a run. The wind was on our back, the spinnaker was up, and we were surfing the waves.

The Death of Ernst Knobil and Devastation from Tropical Storm Allison

After more than 10 years of successfully building the department, tragedy struck. In April 2000, Ernie succumbed to pancreatic cancer. Ernie was my good friend and fellow sailor, and my mentor and adviser on all things related to academic issues and biopolitics. I still feel his absence. A year later, Tropical Storm Allison came to Houston. The basement of the medical school was flooded, and we had one foot of water on the ground floor. Animals were housed in the basement. We lost all 5,000 of them, including 75 primates. The cyclotron and 3T MRI were destroyed. Emergency power was also lost, so the contents of every freezer and refrigerator in the school were destroyed. The school was devastated and so was the department. Some of the crew jumped ship, and I understood why. Jocelyn lost all of her

primates, and it would be years before the primate facilities could be reestablished. She had no choice but to relocate to Emory University, which had the facilities she needed. At a time when the NIH was doubling and floating the expansion of universities, we were in dry dock and there was no growth to be had.

The Loss of Stan Schultz as Dean

All efforts were focused on returning to some semblance of normal. The university decided to never put animals back in the basement, and, as a temporary measure, animals were housed in converted lab space throughout the building. But there was no high ground to be found for a permanent fix. Here a bit of lemonade came from the disaster. The university took the Federal Emergency Management Agency (FEMA) funds, insurance money, and new state funding to construct an extension to the existing medical school building. The top two floors of the new building were devoted to animal housing, leaving the four lower floors for research expansion. Another good sign was the appointment of Stan Schultz as dean of the medical school. Stan planned to use the new space to expand the basic science departments, with each department receiving one full floor. He appointed me as assistant dean for research to provide faculty input on the design of the building. It was a major undertaking, and my responsibilities ranged from major issues, such as the extent of dry vs. wet and dedicated vs. shared lab space, to minor details, such as the selection of carpeting and paint color. After five years of planning and construction, the end result was worth the investment. Sadly, as the building was nearing completion in 2006, Stan became ill and needed to relinquish the deanship. With his departure, support for the expansion of basic science research faded. The wind had shifted and we changed from sailing on a run to sailing on a beat.

Stepping Down as Department Chair

Being a department chair is great when there are resources to grow and build, but things were never quite the same after Stan stepped down as dean. I continued on for the next 10 years making some outstanding new recruitments, such as Michael Beierlein, Valentin Dragoi, and Fabricio Do Monte. But, it was time for me to step down and let others take the helm and push the administrative frontier. I knew the writing was on the wall when, toward the end of one of my annual chair reviews, the dean said: "Jack, you know that you have more time behind you than ahead of you." I responded that "I was just getting started." It was a friendly encounter, but there was a message. There is an old saying in the boating community: "The happiest day of your life is when you buy your new boat. The second happiest day is when you sell it." And as Ernie always said, and as I tell all my colleagues,

"don't worry about administrative ups and downs; concentrate on your research and everything will take care of itself." It was time to leave the leaky ship for a different boat. Fortunately, I always keep two career boats, and the other one was still in great shape and ready to sail to new horizons.

Back to the Science

Empirical Results Were Showing Complex Dynamics of Genes and Proteins Associated with Long-Term Memory

By 2000, it was becoming increasingly clear that waves of changes in gene expression and protein synthesis occur in *Aplysia* and other animals as memories are acquired. When Rong-Yu Liu joined the lab in 2003, we initiated a major effort to quantify those dynamics and, importantly, to understand their functional significance. Rong-Yu received her doctorate in neuroscience from the Shanghai Institute of Brain Research, Chinese Academy of Sciences, and then received postdoctoral training at the University of North Carolina working with William Snider on the signaling pathways underlying axonal regeneration. I have been extremely fortunate that we have been able to continue working together. She is a pro at studying LTS and LTF.

Rong-Yu made the extraordinary discovery that 5-HT induced changes in mRNA and protein levels that had distinct temporal signatures. For example, immediately after 5-HT, levels of the transcriptional activator cAMP responsive element binding protein 1 (CREB1) increase, return to basal levels 1 hour later, and then rebound at 5 hours. Similar rich dynamics, albeit with a different time course, were seen with the transcriptional repressor CREB2. At the same time, it was becoming clear that there were distinct differences in the dynamics of second messengers activated by 5-HT and sensitizing stimuli. As shown originally by Lise Bernier working in Jimmy Schwartz's lab and later extended by Tom Carew and colleagues, cAMP levels increased rapidly in response to a single stimulus, but they did not persist. In contrast, as shown by Kelsey Martin and Eric Kandel, and later extended elegantly by Gary Phillips and Tom Carew, pERK (active, phosphorylated extracellular signal-regulated kinase) is slow to activate. And importantly as Gary Phillips and Tom Carew found, once activated, pERK can be rapidly inactivated.

The Right Time to Learn

These unusual dynamics got us thinking. Perhaps they could help explain the almost universal phenomenon established in 1885 with the seminal work of Herman Ebbinghaus that spaced training with long intertrial intervals is more effective at producing memory formation than massed training with short intertrial intervals. Attempts to optimize the spacing effect generally are based on trial-and-error approaches for choosing the intervals between stimuli. Consequently most, if not all, training protocols used in animal and

human studies are probably not optimal. And different protocols were being used for *in vivo* and *in vitro* studies in *Aplysia*. Four trials had been commonly used for LTS training by Harry Pinsker in 1973 and by Bill Frost in 1985, but Sam Schacher in his 1986 *Science* paper introduced a spaced training protocol for long-term synaptic facilitation (LTF) consisting of five pulses of 5-HT, with 20-minute interstimulus intervals. So LTS training used four shocks, whereas LTF training used five. When I asked Sam at an SfN meeting why he used five trials and not four, without thinking, he said, "I added one for good luck." I am using this anecdote because it illuminates how scientists develop intervals and number of trials for behavioral training protocols. It is all trial and error and a bit of good luck. And once you find something that works, you stick with it. It does not pay to fully explore the parameter space.

Yili Zhang joined the lab in 2008 having done her graduate work in computational biology at Rutgers University where she worked with Jorge Golowash. She also had two master's degrees—one in computer science from Virginia Polytechnic Institute, and the other in pathology from the National University of Singapore. She was well poised to tackle the challenge of determining the optimal training schedules for learning. Based on the differences in kinetics of the PKA and ERK cascades, we hypothesized that learning might be improved if the training trials were in sync with the different dynamics of these cascades. Specifically, protocols that maximize the overlap of PKA and ERK activities should induce stronger LTF. To test this hypothesis, Yili, Doug Baxter, Paul Smolen, and I built a reduced mathematical model to simulate the induction of LTF. In this reduced model, PKA and ERK converge on a phenomenological variable "inducer." It was used to represent the product of PKA and ERK activities, and hence a reflection of downstream gene regulation necessary for the induction of LTF. The greater the peak level of inducer produced by a given protocol, the more likely that the protocol will induce a stronger LTF. We used ordinary differential equations to implement the model and simulated 10,000 different 5-HT protocols to find one that maximally activated inducer. Each protocol had five pulses of 5-minute 5-HT, but the four interstimulus intervals varied from 5 minutes to 50 minutes. We calculated the peak values of inducer in each protocol. The protocol producing the highest peak value of inducer was named the Enhanced protocol. All that was fine, but it was just a model and a simple one at that. Here is where Rong-Yu comes back in the picture. She had become very skilled in making cocultures of sensory neurons and motor neurons and measuring LTF, which could be tracked for at least five days. Rong-Yu tested the prediction using electrophysiological and behavioral techniques. The Enhanced protocol not only induced a stronger LTF than the Standard protocol at 1 day after training, it also prolonged LTF from one day to five days. It increased both the magnitude and the persistence of LTF. Moreover, at the behavioral level, the Enhanced protocol produced stronger and longer-lasting memory for LTS than the Standard protocol.

We also asked if irregularly spaced protocols could enhance normal learning, might modeling also predict protocols that restore learning impaired by a genetic mutation or by other physiological insults? One disorder that impairs learning and memory is Rubinstein-Taybi syndrome (RTS), which is commonly caused by mutations in the gene encoding CREB-binding protein, denoted CBP. This protein is a cofactor in CREB-mediated transcription. Analogs of RTS have been generated by creating a nonfunctional form of CBP in forebrain neurons of transgenic mice. We used siRNA techniques to knock down the expression of CBP and thereby mimicked the deficits in long-term synaptic plasticity found in the mouse models of RTS. Simulations predicted that a "rescue" protocol with irregularly spaced interstimulus intervals similar to the Enhanced protocol would restore LTF, a prediction that was confirmed empirically at the sensorimotor synapse.

Hitting a Home Run

It was remarkable that this simple model had such excellent predictive ability. Nevertheless, it still needs to be expanded to incorporate additional known signaling pathways and transcriptional regulators important for LTF. Moreover, before it can be applied to a mammalian system, the model and its parameters will need to be adjusted to account for the qualitative and quantitative differences in molecular species in different brain areas and different memory systems. On the positive side, tremendous progress is being made in analyzing memory systems and the underlying neuronal and molecular dynamics, which will allow for the development of better models. I have always been encouraged by the words from British statistician George Box: "Essentially, all models are wrong, but some are useful." I believe that the strategy of computationally designed protocols based on the knowledge of the underlying kinase cascades can be extended to explore better training protocols in mammals. When I presented this work in 2012 at a scientific meeting at Scripps Florida, my colleague Paul Benjamin from the University of Sussex said, "we hit a home run." I hope that history will agree and perhaps even call it an out-of-the park grand slam. At the very least, and as Paul Smolen, Yili Zhang, and I argued in our 2016 review in *Nature Neuroscience*, a consensus will emerge that a complete understanding of the spacing effect lies in understanding the dynamics of the molecular cascades involved in memory induction.

Giving Back: A Career in Neuroscience Is More Than Making Discoveries

The paragraphs that follow are among the most valuable in this autobiography because I believe that an important part of being a scientist is educating

younger colleagues and sharing and explaining discoveries with the public. Such activities are particularly important for those of us who have benefited from enormous support from federal agencies, such as the NIH.

Outreach and the Dana Alliance for Brain Initiatives

At the urging of Ernie Knobil, David Low, president of the UT Health Science Center (UTHealth), established the Neuroscience Research Center in 1992. Its purpose was to serve as an "umbrella organization" to bring together neuroscience researchers within UTHealth to promote research, education, and outreach. I was appointed its director. We quickly established a monthly calendar of events, published a newsletter featuring articles from UTHealth neuroscientists, and established an annual neuroscience poster session and a Distinguished Lecture Series. In addition, and inspired by the Dana Alliance for Brain Initiatives, we initiated an annual Public Forum in conjunction with Brain Awareness Week and later Brain Night for Kids. Our local activities caught the attention of the Dana Alliance for Brain Initiatives. I was invited to become a member and participated in some of their national Brain Awareness events. We are all indebted to Barbara Gill and Barbara Best of the Alliance for their enormous support of outreach activities as well as the inspirational leadership of founder David Mahoney. These outreach events were and continue to be extremely gratifying, and they provide an opportunity to give back to the community who, through their philanthropy and taxes, make our research possible.

Summer Courses

An excellent way of interacting with the broader neuroscience community is to participate in summer courses. These events bring together students and faculty for focused efforts on concepts and techniques. I had the great pleasure of participating for many years in the course on Neural Systems and Behavior at the Marine Biological Laboratory (MBL) at Woods Hole and the course on the Biology of Memory at the Cold Spring Harbor Laboratory. Woods Hole was first, when in 1984 course directors Ron Hoy and Eduardo Macagno asked Tom Carew and me to run a two-week lab block on the neurobiology of *Aplysia.* We did this for two years and then another five during the time that Tom and Darcy Kelly were course directors. It was a lot of work, but we were helped out greatly by course assistants Len Cleary, Emily Marcus, and John Walsh.

I think it was a terrific experience for the students. It was also a great time for our families getting together for lunches at the MBL beach and dinners (albeit brief ones because we needed to get back for the "night shift"). The MBL experience had a lasting impact on my children who fondly remember building dams to divert spillover water from the outdoor shower

at MBL beach and playing and riding bikes on Devil's Lane and Memorial Circle. The experience was much more than the physical environment, however. They were constantly exposed to scientists and their families from throughout the United States and abroad, which I am sure influenced their pursuit of career paths in the biomedical sciences.

I also had the great privilege of being codirector for almost 20 years of the summer course on learning and memory at Cold Spring Harbor Laboratory. My involvement started when in 1985, John Koester asked me whether I was interested in taking over his role in a course that he, Eric Kandel, Fernando Nottebohm, and Keir Pearson started on the Cell and Molecular Biology of Behavior. I agreed, but thought the course should be more focused, and with everyone's agreement, changed the name to the Cell and Molecular Biology of Learning and Memory (later changed to Biology of Memory: From Molecules to Behavior). Eric and Keir stayed on as codirectors, and we brought on board Larry Squire to cover memory systems. Later when Eric dropped out, Howard Eichenbaum and Kelsey Martin joined the team. Over the years, we had a fantastic series of lecturers, including, and to name just a few, Cristina Alberini, Tim Bliss, Allison Doupe, Leslie Griffith, Peter Holland, Mary Kennedy, Joe LeDoux, Steve Lisberger, Robert Malenka, Robert Malinow, Roger Nicoll, Chip Quinn, Cathy Rankin, Jennifer Raymond, Robert Rescorla, Christie Sahley, Jimmy Schwartz, Dick Thompson, and Scott Waddell, all leaders in their respective areas. Every summer was an intense update on the latest developments in the field. It was as great for me as it was for the students. And, we had some great students who have gone on to successful careers, including Tobias Bonhoeffer, Edward Boyden, Ruth Colwill, George Dragoi, Barbara Knowlton, Elizabeth Buffalo, and Wendy Suzuki, just to name a few.

Society for Neuroscience

I also became very active in the Society for Neuroscience, serving on numerous committees, including twice on the Government and Public Affairs Committee. It was a great opportunity and honor to represent neuroscience to the public and also to our congressional representatives through the SfN's annual Hill Day events. I also served as treasurer of the society. I "could'a been president," but at least I was a contender on this waterfront. In fact, a two-time contender. Perhaps there was a limit to my good fortune, as I lost these elections to David Van Essen (2006) and Eric Nestler (2015). My losses were disappointing, but as Mort Mishkin once told me "you win if you win, and you win if you lose." And, one benefit of losing is becoming eligible to receive an invitation to join the Presidential Losers Club and attend the annual luncheon held in conjunction with the meeting of the SfN. The regulars at the luncheon, organized by club founder and three-time loser John Hildebrand, include me, Frances Jensen, Julie Kauer, Irwin Levitan, Murray Sherman, Nick Spitzer, and Leslie Tolbert.

Open-Access Neuroscience

One of my proud achievements in teaching was the development of an open-access textbook of neuroscience. The project began in 1999 with the encouragement of William Weems, a former colleague in the Department of Physiology and Cell Biology, who developed computer animations to assist in the teaching of cardiovascular physiology to medical students. He thought that neuroscience could benefit from such an approach as well. I agreed and took the concept up a notch and developed a complete online textbook of neuroscience. It was clear that computer-assisted teaching methods have great advantages over traditional methods. Animations in particular are ideal for teaching neuroscience, given the ubiquitous dynamics associated with neuronal processes, such as action potentials, synaptic transmission, and circuit activity, which in traditional textbooks can only be represented with static images. Also, an online format allows for continuous updates, avoiding the fate of traditional textbooks that become outdated soon after they are published. Bill used his position as associate vice president of academic technology to support the project by putting two graphic artists on the project. Needless to say, I was also fortunate to have the teaching faculty of the department behind the project. We essentially took the medical school neuroscience course and converted it to an electronic format. The project was completed and released as *Neuroscience Online* in 2009. It has been an enormous success and is used by individuals and as a core textbook for neuroscience courses around the world. In 2020, it received more than two million visits.

I have not formed any companies or tried to profit from my discoveries. The privilege of being able to do science and make discoveries is compensation enough. And what a privilege it has been to work with so many exceptional graduate students, postdoctoral fellows, and other colleagues who have inspired me and become my friends. I regret that I could not do justice in this autobiography to their enormous contributions. I also could not have wished for a better animal to work with. I am sure *Aplysia* has many answers to questions that have yet to be asked.

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