Gerald D. Fischbach

Born:

New Rochelle, New York
November 15, 1938

Education:

Colgate University, Hamilton, NY, B.A. (1960)
Cornell University, Ithaca, NY, M.D. (1965)

Appointments:

Senior Surgeon, National Institute of Neurological Diseases and Stroke (1966–1969)
Staff Fellow, National Institute of Child Health (1969–1973)
Associate Professor of Pharmacology, Harvard University (1973–1978)
Professor of Pharmacology, Harvard University (1978–1981)
Edison Professor of Neurobiology and Chair, Department of Anatomy and Neurobiology, Washington University of St. Louis (1981–1990)
President, Society for Neuroscience (1983–1984)
Nathan Marsh Pusey Professor of Neurobiology and Chair, Department of Neurobiology, Harvard Medical School (1990–1998)
Non-resident Fellow of the Salk Institute (1990–2010)
Director, National Institute of Neurological Disorders and Stroke (1998–2001)
Executive Vice President Health Sciences and Dean, Columbia University (2001–2006)
Director, Simons Foundation Autism Research Initiative (2006–2013)
Chief Scientist and Fellow, Simons Foundation (2013–current)

Honors and Awards (Selected):

Mathilde Solowey Award in Neurosciences, NIH, 1975
Honorary M.A. Harvard University, 1978
W. Alden Spencer Award, Columbia University, 1981
President, Society for Neuroscience, 1983
Member, National Academy of Sciences, 1984
Member, American Academy of Arts and Sciences, 1988
Member, Institute of Medicine, 1990
Member, European Academy of Sciences and Art, 1991
The Foundation Ipsen Neuronal Plasticity Prize, 1998
Member, American Philosophical Society, 2003
Honorary Doctor of Science, Colgate University, 2003
Honorary Doctor of Philosophy, Hallym University, South Korea, 2008
Fellow, American Association for the Advancement of Science, 2008
Honorary Doctor of Science, Washington University in St. Louis, 2015

Gerald Fischbach has studied the formation and maintenance of chemical synapses throughout his career. He pioneered the development and use of dissociated nerve cell cultures to study the physiology, morphology, and biochemistry of developing nerve-muscle and interneuronal synapses. He found that at the neuromuscular junction, synaptic transmission begins immediately after a motor axon growth cone contacts a naïve muscle cell, but maturation of this prototypic synapse continues for weeks thereafter. The early appearance and regulation of glutamate and GABA receptors in spinal cord and hippocampal neurons and the modulation of voltage-gated calcium channels in developing neurons was studied as well. Neuregulin, a protein purified from brain tissue based on its ability to promote the accumulation of acetylcholine receptors at nerve-muscle synapses, recently has been implicated in the developing central nervous system and in certain neuropsychiatric disorders. Fischbach is widely recognized as a scientific leader, possessing a deep understanding of the normal and disordered brain. He has served in leadership roles at three universities, at the National Institutes of Health, and at the Simons Foundation. He has helped transform neuroscience in several areas of study, and he has influenced the careers of many scientists throughout this country and abroad.

Gerald D. Fischbach

My long-term interest in neuroscience has been the formation, function, and plasticity of synapses. Most often my research has involved the neuromuscular junction (nmj), the synapse formed by spinal motor neurons on skeletal muscle fibers. Beyond the nerve-muscle synapse, I have studied synapses formed between neurons dissociated from the central nervous system (CNS) and from peripheral ganglia.

What follows is a brief summary of selected findings from my laboratory over the course of the past half-century. This is not an exhaustive review of my work or of the field at the time the work was conducted, or of the current literature. But this memoir is but a start.

The recitation of experiments is not strictly in chronological order. Techniques emerge at different times, personal matters intervene, and some projects proceed more rapidly than others. Rather, I have presented experiments in a more coherent way as they appear to me today.

An important part of my life in science has been the administrative responsibilities that I have held. One might consider these duties as burdensome distractions, but I learned a great deal from each experience. I came to consider science administration, with the possibility of inspiring others, as science on a meaningful level.

One advantage of the nmj is that we know its "purpose": sufficient transmitter acetylcholine (ACh) must be released from the nerve terminals to activate a sufficient number of ACh receptors (AChRs) in the postsynaptic membrane to trigger an inward current sufficient to depolarize the membrane past threshold and trigger a muscle contraction. The postsynaptic membrane at the nmj contains a high density of AChRs. This remarkable specialization became a main focus of my work.

Another advantage of the nmj is the remarkable legacy left by Bernard Katz, Stephen Kuffler, John Eccles, and many others. My generation benefited from their studies of synaptic transmission extending from the dawn of microelectrode physiology to current giga-seal single-channel recordings. The case can be made that most of what we now know about chemical synapses in the brain was either first or best described at the nmj.

Early Personal History

My first four years were spent in Brooklyn, but I grew up in Mount Vernon, New York, a comfortable suburb squeezed between the Bronx and wealthier sections of Westchester County. My parents were children of Jewish

immigrants who arrived from Russia, Romania, and Austria. My mother was a graduate of Hunter College. Her warm, outgoing nature helped her succeed as a leader of Jewish communities, and as a real estate broker in a competitive market.

My father was a lawyer educated at Columbia University and Brooklyn Law School. He was a demanding intellectual, proud of his encyclopedic knowledge of many, somewhat irrelevant, topics. One of his joys was to complete the Sunday *New York Times* crossword puzzle, which he often did in less than an hour. My sister Joan and I tolerated his incessant challenging questions and high expectations. I regret that I did not appreciate him when I could. He had a debilitating neurological disorder characterized by muscle wasting and an intention tremor. As I matured in college and medical school, he had difficulties with his law practice that embarrassed him and presented a further block to our communication. I have kept several of his books and continue to be impressed with the diversity of his interests.

Joan, four years my senior, pursued various careers, including occupational therapy and, like my mother, real estate sales. She moved to Denver 40 years ago, and we grew apart. This emotional distance, thankfully, has been reduced in recent years. Joan cared for both my mother as her memory failed, and my father as his neurological deficits progressed.

The 1940s and 1950s in Mount Vernon are a blur to me, as they are to many who grew up in that era. We were innocents who managed to ignore the gloom of the Cold War, the legacy of the holocaust, racial segregation, and the threat to civil liberties. I was a good, but not exceptional student in high school.

I left Mount Vernon in 1956 to attend Colgate University on a New York State Regents Scholarship. This small stipend of $2,500 covered tuition at that time. It was at that small liberal arts college in the beautiful Chenango Valley in upstate New York that I developed a deep self-confidence and began to enjoy academic pursuits.

I became a freshman dorm advisor in my second year, helping new students about my own age adjust to academic and social challenges. This early experience played a role in the satisfaction I derived from organizing diverse groups in academia as a student, lab chief, department chair, institute director, and dean. I excelled academically at Colgate, graduating magna cum laude with high honors in mathematics and chemistry and was accepted early decision at Cornell Medical College with new scholarship support from New York state.

Cornell University Medical Center (1960–1965)

The Cornell medical faculty was excellent at that time with Vincent du Vigneaud who isolated and sequenced oxytocin, Harold Woolf, Fred Plum, and Jerome Posner working in my areas of interest.

Marriage

I met Ruth Zeitlin in 1960 during our first weeks at Cornell, and my life changed forever. Ruth had begun a three-year program at the Cornell University–New York Hospital School of Nursing after completing two years at Mount Holyoke College. We moved imperceptibly from acquaintances, to study partners, to close friends, to an engaged couple. We married in 1962 despite warnings from our parents that we were acting precipitously. What if Ruth became pregnant? We assured them of our maturity in such matters and then proceeded to have two wonderful children in the next two years. Elissa was born in 1963 and Peter in 1965. We lived in a small, fifth-floor walk-up for two years. Mark and Neal, our unsuspected twins, were born three years later when we lived in Bethesda, Maryland. Perhaps because of our early indiscretion, we now have 12 wonderful grandchildren. These events were and remain the most important of my life. We still celebrate the union of four wonderful families: Wolsk, Zeitlin, Fischbach, and Coopersmith. Three years ago, we purchased the home in Purdys, New York, once owned by Ruth's parents, where we were married in 1962.

Despite the challenges of raising four children and attending to a needy husband frequently on the move to different parts of the country, Ruth forged a remarkable career creating important programs at Washington University, Harvard, the NIH, and Columbia University, completing a PhD in medical sociology and a graduate degree in psychiatric epidemiology along the way. Her enduring interest has been in the field of bioethics. She created and has served as director of Centers of Bioethics at Washington University and at Columbia. Ruth gave me the courage to take a leave of absence from medical school to pursue laboratory research and to make every subsequent move over the years.

I tolerated much of the memorization required in medical school at that time because the subjects were interesting. But rote learning was a far cry from the independent honors work I completed at Colgate, and I was soon bored. One lab exercise stands out in my mind. We were charged with recording the resting membrane potential of frog Sartorius muscles. I recorded a value of −90 mV, the highest value in the class, probably aided by a tip potential of a poorly constructed microelectrode. In any case, the rush of success reinforced my urge to break out, and with Ruth's encouragement, I decided to take a year off from medical school to learn electrophysiology. Few students took this option in 1962.

I spent the year in the Department of Pharmacology. Walter Riker, the empathetic chair, provided me with a room of my own, equipment, and an interesting question: Does ACh, the transmitter of excitation from motor axons to postsynaptic muscle fibers also exert actions on presynaptic motor nerve terminals? I was on my own, an independence that gave me the opportunity to study the literature of synaptic physiology and the

electronics required for intracellular microelectrode recording. Meetings with Ernie Amatniak, Harry Grundfest's former technician, at his company Bioelectrics, taught me a great deal about high-input-impedance unity gain amplifiers.

I was proud that my work on quantal release of ACh from motor nerve terminals treated with an anticholinesterase was awarded the Harold G. Woolf Prize at Cornell. Less important was the rejection of my single-authored manuscript by *Science* magazine. I had the bug, and I wanted to continue in neuroscience research. An important conversation with Eric Kandel, a cousin by marriage, convinced me to apply to the Laboratory of Neurophysiology at NIH for further training. Kandel had recently completed a successful tour in that laboratory where his groundbreaking work with Alden Spencer on the hippocampus shaped his long-term interest in synaptic plasticity and memory. Kandel and I began a conversation about neuroscience that has lasted, on and off, for 50 years. I was accepted for a position at the NIH in the Laboratory of Neurophysiology directed, at the time, by Wade Marshall. Phil Nelson directed the Spinal Cord Secton of the lab, the unit to which I applied.

There were no openings for one year, so I accepted a medical internship at the University of Washington Medical Center (UWMC) in Seattle. The UWMC was growing rapidly in 1965. There was great expertise in infectious disease, led by Robert Petersdorf, the chair of medicine; in renal disease with the first kidney transplant; and in neuroscience. Bertil Hille and Charles Stevens were members of the Physiology Department, and I naively thought I would have plenty of time to meet and perhaps work with them during the year. Seattle was a welcome change for Ruth and for me. We loved to camp, hike, and ski, and we still describe a beautiful day in the East as a "Seattle day."

I enjoyed the internship experience, and I was good at it. Secure in clinical medicine, I planned to return to New York City for advanced training in neurology with Fred Plum and Jerry Posner at Cornell after I served time in the Public Health Service at the NIH. It took me several years to turn away from this plan.

An important event that reinforced my decision to join the Public Health Service without delay was a telegram from my draft board requesting that I report within three days for duty in Vietnam. Ruth called to inform me of this request at 3:00 a.m. while I was working at the hospital. The "doctors draft" during the Vietnam War was indeed a significant factor in creating the superb community of young biomedical scientists at NIH during those years.

National Institutes of Health (1966–1973)

In 1966, the NIH sat in a bucolic, wooded campus of more than 300 acres in Bethesda, Maryland. The Laboratory of Neurophysiology spanned the

National Institute of Neurological Disorders and Stroke (NINDS) and the National Institute of Mental Health (NIMH), a wonderful precedent that is echoed in the new intramural John Porter Neuroscience Center that brings together scientists from several institutes. Senior investigators, including Phil Nelson, Karl Frank, Mike Fuortes, Ed Evarts, Will Rall, Ichi Tasaki, Walter Freygang, Milt Bridgman, and Marshall Nirenberg, interacted seamlessly with more junior scientists, including Bob Burke, Bob Wurtz, Dennis Baylor, Mahlon DeLong, Tom Thatch, Al Gilman, John Heuser, Tom Reese, and Stan Rappoport. I was welcomed into the midst of this extraordinary community. They offered an exciting, often iconoclastic, outlook on important problems in brain science and shared a great passion for research that shaped my life in science.

These early years at the NIH continued my march toward independence. The scientists were friends and mentors for each other. But in the main, I was on my own with ample resources and enough time to think about the questions I asked and to explore new techniques. I realize in writing this memoir that nearly all of my subsequent ideas and experiments can be traced back to this extended postdoctoral experience in Bethesda.

Use and Disuse at the nmj

My first experiments at the NIH, undertaken with Norman Robbins, focused on effects of use (exercise) and disuse (immobilization) on the contractile properties and synaptic function of skeletal muscle fibers. We took use and disuse as a better model for "experience" or "learning" than was denervation, a popular paradigm at the time. Robbins was more experienced in the lab than me, having studied trophic interactions at Rockefeller University with Benice Grafstein and Paul Weiss, but he treated me as an equal partner from the start.

Postural muscles such as the soleus in the calf are tonically active, and their twitch time is slow. When the hind limbs of rats were immobilized, the pattern of activity, measured with indwelling electromyography (EMG) electrodes, changed from low-frequency tonic activity to high-frequency bursts characteristic of fast-twitch muscles. Immobilized, tonic muscles developed faster contraction times, higher tetanic fusion frequencies, and lower twitch–tetanus ratios. The discovery that properties of innervated muscle fibers were not fixed was a revelation. As a model for the brain, it emphasized the role of experience in molding the properties of target cells.

The pattern of motor neuron activity also altered the distribution of AChRs in the muscle membrane as measured by iontophoretic application of ACh. This was the beginning of my interest in AChR regulation by impulse activity or by neurotrophic influences.

Beyond our immersion in medical science, the NIH group was socially and politically active. Robbins introduced me to a challenging brand of

political activism, a lifelong commitment of his. Remarkably, it was possible to pursue one's conscience at the NIH in the late 1960s and early 1970s. I joined the Physicians for Social Responsibility, participated in demonstrations at the NIH and at the capital, and appeared on television talk shows. Ruth joined Bella Absug's Mothers Strike for Peace. On one occasion, they tried to surround the Capitol building in a human chain. Our daughter, Elissa, who was seven at the time, remembers fearing her arms were being pulled from their sockets as the line was stretched. How different is the situation today.

Leap into Cell Culture

After a productive collaboration with Robbins, I wanted to develop a preparation in which I could observe the sequence of events during nerve-muscle synapse formation in real time. Cultures of dissociated cells offered many advantages, including direct visualization of fine neurites in unfixed preparations by phase contrast or interference contrast microscopy, and precise control of the microenvironment of the developing cells. Looking ahead, I realized that sparse cell cultures would provide convenient assays for studies of known neurotrophic factors and for purification of new ones.

From the start, my intent was to prepare co-cultures of motor neurons and muscle cells, but I cut my cell culture teeth working with muscle cells grown alone. The electrical properties of excitable cell membranes are exquisitely sensitive, and they seemed like a good bioassay for health in vitro. I was lucky enough to meet Mark Nameroff then at the Walter Reed Hospital. Nameroff was a student of Howard Holtzer, one of the pioneers in studies of myogenesis.

We plated myoblasts dissociated from chick pectoral muscles cells in Nameroff's lab. After the mononucleated myoblasts settled on the collagen-coated tissue culture dishes and had begun to fuse to form multinucleated myotubes, I drove them to my electrophysiological rig at the NIH in a lidded container wedged upright on the floor of my Ford station wagon. The loss of CO_2 was not severe, provided the traffic was light, and the cells did not complain loudly.

We estimated membrane resistivity and capacitance by solving the second-order partial-differential cable equation assuming that the myotubes were closed-end cylinders. I was happy and relieved to find that the membrane resistivity, 2,000 ohms cm^2, and the specific capacitance, 1 $\mu F/cm^2$ were comparable to values of muscle fibers in vivo. Cultured myotubes cells were "tight" rather than leaky sacks.

Wilfred Rall recently had published his multicompartmental model of branched motor neuron dendrites as equivalent cylinders that revolutionized our thinking about dendritic arbors. Rall was a great help to me as I modeled branched myotubes to determine their specific membrane

properties. The classical papers of Rushton on the cable properties of nerve axons were also a great help.

The resting membrane potential of myoblasts and young myotubes was low, but it increased with time in culture, and the cells developed overshooting action potentials. Many fibers twitched vigorously and many exhibited spontaneous tetanic contractions. I believe that this study was the first quantitative description of development of electrical excitability in vitro of any cell type.

Tom Smith loaned me my first inverted microscope. After I pledged to care for it with my life, I picked up the heavy Zeiss scope by the arched neck and watched, helplessly, as the heavy, dependent optics rolled off the rack and pinion and fell to the cement floor. I was forgiven by Smith, always the generous scholar, and we soon collaborated on studies of spherical "myoballs" created by treatment of the cylindrical myotubes with colchicine. We used this preparation to measure voltage-gated Na, Ca, and Cl currents under voltage clamp conditions.

Nerve-Muscle Synapses Form In Vitro

I moved on to co-cultures of dissociated spinal cord and muscle. My first publication described the fact that nerve-muscle synapses, in fact, do form in sparse cell culture. This was revolutionary at the time. I vividly recall the thrill I felt sitting alone in my small, dark physiology room listening to the hum of vacuum tube amplifiers and staring at the glow of the oscilloscope screen when the first synaptic potentials appeared. The excitement and beauty of the moment are with me today.

My resolve to return to medicine was shaken, but the decision was drawn out. I enjoyed working with patients, and in later years, I returned to clinically relevant problems. Our parents had experienced the Great Depression and saw a more secure future for us in clinical practice. They were concerned, but they did not apply pressure. As always, Ruth urged me to think about the moment and to emphasize the positive. We bought and renovated an old Sears Roebuck–designed house in Potomac, Maryland, mowed two of our four acres, boarded horses, raised white leghorn chickens (hatched, inadvertently, in my lab incubator), and continued to focus on our ever more active kids.

Earlier in the year, I agreed to substitute for K. Frank as the project officer on a PL 480 grant to Kamal and Susheel Sharma in the Department of Physiology of the new St. John's Medical College in Bangalore, India. Before leaving, I wrote a "brief report," as science papers were called at the time, and mailed it off to *Science*. I heard nothing about the paper while we spent four months in India. When I returned to the NIH, I found the manuscript on my desk returned for "inadequate postage." When I recovered, I added a few stamps, and sent it off again. The paper was accepted by return mail.

Bangalore was an interesting, transforming experience for us all. I went to work every day at the medical school and at the nearby Raman Institute, Ruth worked in the hospital, and Peter and Elissa attended local schools and made friends. The tapping of the night watchman's stick on the path outside of our apartment to chase cobras into their nests, and Ruth's warning not to slam the refrigerator door and disturb the sediment in our water jugs, remain in our thoughts. But that is another essay. Mark and Neal, who were two years old at the time, waited for us at home in the loving arms of their grandparents.

On our return from India, I began immediately to expand the work on nerve-muscle synapse formation in vitro. I was joined by Stephen Cohen, then a medical student at Georgetown University. We further simplified the neuron-muscle cultures by eliminating fibroblasts, which in some situations are unwelcome "contaminants." Even the finest neurites could then be visualized and followed over long distances as they contacted nearby and more distant myotubes. Our preplating technique followed by treatment with antimitotic agents resulted in essentially "pure" nerve-muscle co-cultures, and many labs adopted this strategy.

The mean quantum content of evoked epps and the mean mepp frequency were low at nerve-muscle synapses identified between 4–25 days of co-culture, and many muscle fibers were innervated by more than one axon and at more than one site. Moreover, synaptic potentials decayed slowly, and they were not further prolonged by physostigmine, implying the absence of acetylcholinesterase (AChE). This conclusion was confirmed in subsequent experiments with Lee Rubin at Harvard by focal extracellular recording of slow synaptic currents and by the absence of staining for AChE reaction product. AChE does not appear when the cells are grown in the presence of blocking concentration of curare or local anesthetics. AChR clusters do appear under the same conditions. Thus, AChE appears at developing junctions later than the initial accumulation of AChRs, and it appears to depend on the level of nerve-muscle activity.

Three questions raised in these early experiments remain unanswered to this day. First, direct electrical coupling between synaptically connected motor neurons–myotube pairs was observed on several occasions. Do such contacts play a role in the initial stages of synapse formation? Second, a degree of specificity in nerve-muscle synapse formation was evident. Not every contact between a competent cholinergic neurite and a receptive myotube resulted in a functional synapse. What is missing at nonfunctional contacts? Third, why is the appearance of AChE at synapses delayed?

Most important for my future work was the observation that regions of high AChR density, 5–20 × background levels were identified on innervated myotubes by focal ACh micro-iontophoresis. In the first experiments, we knew the muscle fibers were innervated, but the precise relation between such hot spots and the overlying neurites was not determined. This was accomplished in subsequent experiments by focal extracellular stimulation

and recording. The same estimate of synaptic–extrasynaptic AChR density ratio was obtained by ^{125}I-αBTX autoradiography. AChR clusters were identified by electron microscopy (EM) after labeling with ferritin-coupled αBTX and by the appearance of intramembrane particle clusters in freeze fracture EM images at physiologically identified hot spots.

To our great surprise, we found similar ACh hot spots on uninnervated myotubes. This finding raised the important question whether neurite associated hot spots were induced or sought out by the incoming motor axon. Subsequent experiments at Harvard showed that neurite-associated receptor patches were induced by motor axons. Moreover, we found that new synaptic ACh aggregates contained newly synthesized receptors.

The extrasynaptic "background" ACh sensitivity on innervated muscle cells was essentially the same as that on uninnervated myotubes even after two weeks of co-culture. It took muscle activity—either spontaneous or driven by direct electrical stimulation—to lower sensitivity. Synaptic AChR clusters remain under these conditions, underscoring our belief that the nerve terminal must exert an influence to preserve synaptic receptors. Accumulation of AChRs at developing nmjs in vivo also occurs in the face of increasing muscle activity. It was natural to think of a locally acting trophic influence of nerve on muscle, a thought that occupied me over the next decade.

Many AChR aggregates on innervated and uninnervated myotubes were located immediately adjacent to muscle nuclei. This was an early hint that synaptic receptors were newly synthesized molecules. Jumping ahead in this narrative several years, additional evidence for the appearance of new AChRs came from measurements of AChR subunit mRNAs and from differential labeling of preexisting and newly inserted receptors. Fluorescence photobleaching recovery (FPR) measurements with Janet Dubinsky and Elliot Elson at Washington University showed that new AChRs were inserted centrally within receptor patches under neurites and not solely around the perimeter of such densely packed receptor clusters.

There was much to learn about neurons dissociated from embryonic spinal cord, brain, and peripheral ganglia. Neurons dissociated from E4–E7 spinal cords matured rapidly in sparse cell cultures. They generated action potentials and formed both excitatory and inhibitory chemical synapses with one another. Marc Dichter, newly arrived from New York University and his studies of epilepsy with Alden Spencer, was a great help in these early experiments. Subsequent studies of glutamate and GABA-mediated synapses and modulation of voltage-gated Ca channels were conducted at Harvard and Washington University.

Harvard (1973–1980)

I missed the breadth of a university environment and I enjoyed teaching, so despite all of the advantages of the NIH, after eight years, and much

thought, I accepted a position in the Department of Pharmacology at Harvard Medical School. Irving Goldberg, a biochemist interested in antibiotics, was the chair. He had recruited a small but excellent faculty. The department had a distinguished history. Otto Krayer, the previous chair, came to this country after he was banned from all German universities for refusing to occupy a professorship from which a Jew was forced to resign on ethnic grounds. Krayer had perfected the heart-lung preparation for his own studies, and he built a department with expertise in cardiovascular pharmacology, addiction research, calcium metabolism, pharmacokinetics, and neuroscience. Indeed, Steve Kuffler moved to Harvard to join Krayer's department in 1960 and was soon joined there by David Hubel, Torsten Weisel, Ed Furshpan, David Potter, and Ed Kravitz. This group split off five years later to form the Department of Neurobiology.

Krayer was disappointed with Harvard because of this split and other decisions that adversely affected the department that he had built over many years. He left Harvard and did not return until 1978 when Bernard Katz delivered the first Otto Krayer Lecture.

Kuffler was an important influence in my decision to move to Harvard. We first met at an SfN meeting in 1972, and we remained friends until his untimely death in 1981. Tennis, the Marine Biological Lab, and the nmj were our enduring shared interests. Kuffler had a genius for identifying simple preparations that allowed application of elegant techniques to answer important questions in synaptic biology. I was influenced by his work on the microphysiology and anatomy of the nmj. He was interested in our work on synapse formation in vitro. I also was influenced by the ongoing studies of Edwin Furshpan, David Potter, Story Landis, and Paul Patterson on the fate of autonomic ganglion neurons in sparse cell culture. They showed that the tissue culture environment influenced the transmitter synthesized by these neural crest derivatives. This remarkable, unexpected finding expanded our view of plasticity in developing neurons.

Among my first impressions of Harvard Medical School was the elegance of the Quadrangle, bordered by four classic marble research buildings with the elevated administration building at one end overlooking a spot of green in the midst of semi-urban Boston. Countway Library, a symbol of history and tradition in biomedical science, stood off to one side. Harvard University together with its affiliated hospitals was and remains to be one of the premier medical communities in the world.

We lived in Brookline in a handsome three-story house that Ruth discovered en route to the airport after a long day of house hunting. A large yard was great for the kids especially during the blizzard of 1978, when they constructed igloos (with my prize set of chisels) and small ski jumps. Our nearest neighbors, the Stroms and O'Briens, also members of the Harvard faculty, became close friends. Sinai Temple, a small congregation at Coolidge Corner, offered a meaningful experience at that time in our lives.

Jonathan Cohen joined the Department in 1975. Cohen came from the Institut Pasteur where he worked with Jean-Pierre Changeaux on Torpedo nicotinic AChRs and associated proteins. We met in 1972 during a biophysics summer school in Erice, Sicily. He remembers presentations by Bernard Katz, Rene Couteaux, and me (fine company) and thought it would be nice to collaborate. We did collaborate, eventually, on rapid Na flux measurements in cultured myotubes, and, years later, on the localization of neuregulin receptors at the nmj. Richard Zigmond arrived in 1975 from Les Iversen's lab where he began his important work on tyrosine hydroxylase and catecholamines. Cohen, Zigmond, and I formed the core of our neuropharmacology group.

We organized the neuropharmacology part of the Medical Pharmacology Course. Others involved in that important course included Doug Waud, Peter Dews, Bill Morse, Roger Kelleher, Peter Goldman, Bob Rando, and Irv Goldberg. Waud's emphasis on quantitative drug–receptor interactions, his encyclopedic knowledge of drugs, and his deep skepticism of the pharmaceutical industry were inspiring to students and to me. We also taught neuropharmacology to graduate students, and undergraduates at Harvard College.

During this period, Harvard was among the leaders in reform of the medical curriculum, determined to reduce the tedium of rote learning and to provide more opportunity for hands-on research experiences for students. In addition, four societies were created to bring together students at different stages of their education to share experiences and explore subjects of common interest, including current topics in biomedical science, along with a strong dose of medical history. I enjoyed and contributed to all aspects of these education reforms, and I became the founding master of the Fuller Albright Society.

Pharmacology was close, but not too close, to the Department of Neurobiology. I was welcomed in neurobiology activities, and I taught a course on developmental neurobiology with Patterson for six years. These facts are worth mention, as neurobiology did not welcome many outsiders in their crowded seminar room. Was this only a matter of space? In any case, such isolation eventually worked to their detriment. After Kuffler died and Torsten moved to New York City, the department stagnated. When I returned (from Washington University) in 1990 to chair the department, they had offered only one promotion to tenure in 26 years.

The Marine Biology Laboratory

Kravitz invited me to teach in the neurobiology course at the Marine Biological Laboratory (MBL) in Woods Hole, a job I loved and that became a major influence on my career and our family over the next 20 years. I shared responsibilities for the development section of the course with Story Landis. The talented students included undergraduates, postdoctoral fellows, junior

faculty, and full professors seeking new approaches to their own studies. Students and faculty initiated much original research during the course. Softball, tennis, and swimming were mixed with four-hour lectures and late nights in the lab.

We lived in a small, rented MBL cottage each summer that was packed with our kids and their friends; our dog, cat, and fish; and with visiting cousins and grandparents. After five years, we bought a small house nearby and, then in 1982, after moving to St. Louis, we bought a home on a point of land jutting out into Buzzards Bay. Over the years, we renovated and rerenovated this house. It was a family project with major input from Mark, our architect son, and Elissa, our interior designer daughter. It was Ruth's vision that brought us to this beautiful location, where we lived for the next 30 summers and where we gathered for family vacations and long weekends during the winter. It was her landscaping genius that made our Penzance Point home the most loved and valuable property that we have owned.

Nerve-Muscle Synapse Formation (continued)

Work on the formation of nerve-muscle synapses continued with the help of talented students and postdoctoral fellows. Our lab meetings were full of data and excitement. We demonstrated that AChRs were clustered precisely at newly formed synapses by focal extracellular recording and stimulation, but we still did not know whether competent axons sought out preexisting clusters or induced new ones. We approached this question by first increasing the likelihood of identifying competent axons before and after they formed functional contacts. The most successful procedure was to add small fragments of E4 ventral horn tissue to preplated myotubes. This maximized the number of cholinergic axons in the outgrowth zone and the rapid formation of nerve-muscle synapses close to the explant.

The next step was to follow growth cones as they contacted and innervated myotubes. Eric Frank developed a semi-automated mapping protocol in which the iontophoretic electrode tip was photographed immediately after the optimal response was obtained at each site. In this way up to 50 sites could be mapped at one sitting, and the same site could be relocated hour after hour and day after day. Hot spots were mapped with a resolution of less than 5 microns. The answer was clear. New AChR clusters appeared at new synapses. Noncontacted clusters near a developing synapse often disappeared with the onset of synaptic transmission. Perhaps the fluidity of the membrane in the vicinity of the synapse is altered. An increase in AChR mobility near growth cones was, in fact, found some years later by fluorescence photobleaching recovery experiments at Washington University in collaboration with Janet Dubinsky and Eliot Elson.

Uninnervated as well as innervated muscle cells near the explant contained more membrane AChRs and many more AChR clusters than

did uninnervated muscle cells located farther away in the same culture dish. These powerful images added to our conviction that AChR-inducing substances emanating from the spinal cord tissue were at work.

Immobilization of AChRs within clusters does occur. Indeed, aggregation of preexisting surface at newly formed Xenopus neurite–myocyte contacts was demonstrated by Monroe Cohen, and by other workers in mammalian co-cultures. We found that newly synthesized receptors in chick and mammalian myotubes contribute to new clusters as well. They are distributed throughout the cluster from the start rather than aggregated around the edges.

When is a growth cone "competent" to form a synapse? We evoked the release of ACh from growth cones by focal depolarization within minutes of contact with a myotube. We found that growth cones can release ACh even before muscle contact. A novel "patch-sniff" bioassay was developed in which an outside–out patch of AChR-rich muscle membrane was placed adjacent to a growth cone as it emerged from the cell body and migrated over the tissue culture surface. When the neuron was stimated, a shower of channel openings was evident.

Transmitter release occurs early, but the entire process of synapse formation is a drawn-out affair. In the first weeks after birth, the mean quantal content of evoked endplate potentials increases dramatically, the mean AChR channel conductance increases, the mean channel open time decreases, and the half-life of AChRs in the surface membrane increases from less than 24 hours to more than one week.

Steve Schuetze in my lab initiated studies of ACh channel conductance and mean channel open time and metabolic stability of synaptic and extrasynaptic receptors in vitro and in vivo. He found that the mean channel open time of AChRs on chick and rat myotubes was 4 msec compared with 1.5 msec at mature endplates. The channel properties at rat junctions changed during the first week after birth. And the half-life of receptors in the surface membrane increased dramatically from 18 hours to several days. The properties of chick receptors did not change over the same time course. I believe that the ease of working with dissociated muscle cells and neurons in cell culture facilitated the entry of many scientists to the patch clamp industry.

Schuetze, an extraordinary student and scholar and friend, was tragically killed in a bicycling accident years after he left my lab while on vacation from Columbia University with his wife and friends.

The Search for an Acetylcholine Receptor-Inducing Activity

We began the search for an acetylcholine receptor-inducing activity (ARIA). A plunge into biochemistry was the obvious next step. Tom Jessell, newly arrived from studies with Leslie Iversen, was important in these early experiments. Jessell was fearless and enthusiastic, and his broad critical knowledge of biology was as remarkable then as it is today.

Together with Ruth Siegal, we found that saline extracts of embryonic chicken brains increased the total number of AChRs and the number of receptor clusters on cultured chick myotubes. The activity was destroyed by trypsin, and it was selective in that overall protein synthesis was not changed. A similar activity was secreted into the medium by cultured spinal cord cells. We presumed, of course, we had the factor responsible for inducing synaptic AChR clusters in these extracts. We made regular trips to a slaughterhouse in South Boston, put on boots, and collected heads falling from slaughtered chickens. Fortunately, we soon discovered a source of dissected and frozen chick brains.

While at Harvard, we refined the bioassay and applied several new purification steps. Myoblasts were plated in 96 well plates so each point could be measured in quadruplicate. The rate of appearance of new ^{125}I-BTX binding sites was measured after blocking all surface sites with unlabeled BTX. Precise dose response curves then could be constructed, which showed a marked increase in the rate of AChR incorporation with no change in the rate of degradation. A large percentage of the receptor-inducing activity was soluble in 2 percent trifluoroacetic acid (TFA) and was further purified by reverse-phase high-pressure liquid chromatography. Susan Leeman was a great help at this stage as she shared her expertise and reagents for peptide–protein purification.

Efforts at purification were continued at Washington University. A 42 kD glycoprotein, purified 1.5 million fold, was effective at 10^{-9} M concentrations. We named this protein ARIA, for its acetylcholine receptor-inducing activity. Ted Usdin, an MD/PhD student, deserves great credit for developing and perfecting several new (to us) biochemical steps in this series of experiments.

Final purification of ARIA and isolation of a full-length cDNA was published in 1993 after I returned to Harvard. Doug Falls, David Harris, and Ken Rosen, postdoctoral fellows at the time, played key roles in this effort. The cDNA showed that the purified 42 kD ARIA was the extracellular domain of a large transmembrane precursor protein. The extracellular domain contained an Ig-like domain and a 55 amino acid EGF-like domain that we showed was the active part of the molecule in inducing AChR synthesis. Was this the low-molecular-weight activity detected in our first attempts at purification 10 years earlier? We subsequently found that pro-ARIA is cleaved near the cell membrane and the extracellular fragment binds to heparin sulfate proteoglycans in the extracellular space, probably via the Ig domain.

It was immediately obvious that chick ARIA is homologous to the rat Neu differentiation factor and human heregulin, ligands for the family of erbB receptor tyrosine kinases. Recombinant ARIA, in fact, did induce tyrosine phosphorylation of a 185 kD muscle protein. ARIA is also homologous to glial growth factor (GGF), a protein that was also purified and sequenced at about the same time based on its ability to stimulate the proliferation of Schwann

cells. The science community adopted the name "Neuregulin" (NRG) for this family of proteins. Subsequent work at Washington University, Harvard, the NIH, and Columbia addressed the mechanism of action of Neuregulin (NRG), its likely relevance at the nmj, and its actions in the brain.

Neurotransmitter Receptors in Spinal Cord and Sensory Neurons

During this time at Harvard, we also investigated the GABA responses of spinal cord neurons, GABA-mediated synaptic potentials, and the modulation of submaximal GABA responses by benzodiazepines. Denis Choi and David Farb took the lead in this series of experiments. We discovered that submaximal GABA currents were enhanced by chlordiazepoxide, an allosteric modulation of GABA receptors that had not been described previously. We suggested that such an action might account for the anti-anxiety properties of benzodiazepines, soon to become the most commonly prescribed drugs in America, but were criticized by reviewers because there was no evidence that chickens experienced anxiety.

Kathy Dunlap, Alcmene Chalazonitis, and Ann Mudge studied dorsal root ganglion (DRG) neurons. Thanks to NGF and high K, these cells survive well in sparse cell cultures. Kathy explored the Ca component of DRG action potentials. GABA, noradrenaline (NA), and serotonin (5-HT) all decreased the Ca^{2+} component of the soma spike, which pointed to roles in presynaptic inhibition. Enkephalin also inhibited the DRG Ca spike. This inhibition was accompanied by a decrease in substance P release, consistent with modulation of nociceptive inputs at the spinal cord level. Richard Tsien had studied modulation of Ca currents in cardiac muscle, but our work was among the first to demonstrate modulation of voltage-gated ion channels by neurotransmitters in neurons. This was indeed a productive period in my lab.

Washington University (1980–1990)

After 15 years of intense focus in my own lab, I had begun to think about a wider sphere of responsibility when I was offered the opportunity to chair the Department of Anatomy and Neurobiology at Washington University. Max Cowan, the former chair, had built a strong department based on new neuroanatomical tracing techniques. Modern aspects of cellular and molecular neurobiology did not advance at the same pace. With strong encouragement from Herman Eisen, who had spent 18 years at the university, and from members of the university faculty, including Cuy Hunt, Phil Needleman, and David Kipnis, we decided to uproot our family and move to the Midwest. Elissa was off to college. Peter, Mark, and Neal were ready for something new.

Many factors went into the decision. Mainly, I was attracted by the distinguished academic history of the university and by the excellence of

the current faculty. In addition, we were impressed with the calming beauty of suburban St. Louis. I frequently rode my bike from our home in Clayton across Forest Park, past the Museum of Fine Arts, to the Medical Center. A significant rebirthing of downtown St. Louis was under way, spreading out from the inspiring Arch along the Mississippi River. The symphony conducted by Leonard Slatkin was excellent, and it was accessible. The St. Louis Cardinals were thrilling. Hot humid summers were "tolerated" from our home in Woods Hole.

Dean Kenton King and Vice Chancellor Sam Guze led the Medical Center. King was a quiet leader who demonstrated over and over again how the faculty could achieve great things by working closely with rather than at the expense of others. Guze was chair of psychiatry with a strong sense of the medical school's academic mission. William Danforth, president of the university, was formerly vice chancellor for medical affairs, and he remained deeply interested in medical science. His love of the university and St. Louis had a great influence on me. I had not experienced the same degree of cooperation before coming to Washington University, and I have not enjoyed it as consistently ever since. This community made my move into more administrative duties easy and rewarding. I did not negotiate a start-up package as I was convinced that the school's leadership would do everything possible to help me succeed. And they did.

The preclinical and clinical department chairs met each month in a forum called the Executive Faculty to debate issues of importance to all. They were meaningful and candid debates that taught me a great deal about American medicine and about constructive interactions with partner hospitals.

Ruth and I took part in university intellectual life beyond Medical Center. We attended regular soirees at the home of Eli and Lee Robbins along with members of the English, philosophy, and history departments. Our neighbors in Clayton, Att and Bob McDowell, who was formerly chair of mathematics, brought us even closer to the Arts and Sciences on the main campus. Extensive conversations with Viktor Hamburger in my office and at his home in University City gave me his personal view of neuroembryology, beginning with the embryonic organizer experiments of Hans Speemann and Hilde Mangold, his own early experiments on programmed cell death in the nervous system, and his early collaboration with Rita Levi-Montalcini.

Among those I recruited to the Washington University faculty were Larry Salkoff, Paul Taghert, Jeanne Nerbonne, Andres Burkhalter, Karen O'Malley, and Glen and Jane Phillips Conroy. Glen and Jane are physical anthropologists who carried on the tradition of comparative anatomy in medical school teaching established by the original department chair, Robert Terry, and Mildred Trotter. They became perennial Teachers of the Year. Josh Sanes and Jeff Lichtman joined the department after Cuy Hunt stepped down. And I helped recruit Joe Henry Steinbach and Chris Lingle, bot superb biophysicists, to the Department of Anesthesiology. Dale

Purves, a talented neuroscientist and author, left to chair the Department of Neurobiology at Duke.

I became the founding director of the McDonald Center for Cellular and Molecular Neurobiology and, with time, I also directed the McDonald Center for Higher Brain Function. These centers played important roles in bringing faculty together across disciplines and across the basic and clinical departments, a theme that I promoted later at Harvard and Columbia.

Further Studies of Muscle and Neuronal AChRs

As noted earlier, several studies of AChR accumulation in ARIA- and NRG-treated myotubes were performed during this time at Washington University. At the same time, experiments on muscle AChR clusters induced by ciliary ganglion neurons were initiated by Lorna Role. AChRs accumulate at about 80 percent of the growth cones during the first 24 hours after muscle cultures are seeded with this relatively pure source of cholinergic neurons. Double labeling techniques showed that 80 percent of these receptors were "new"—that is, they were not in the surface membrane before innervation during the first 48 hours of co-culture. Kathy Engisch found a sevenfold increase in nicotinic $\alpha 7$ AChRs in chick ciliary ganglion neurons between embryonic days 6 to 18. The increase occurred despite ablation of the ganglions' target (eye) or inputs (accessory optic nucleus).

Regulation of Glutamate and GABA Receptors

In parallel with our studies of muscle and neuronal nicotinic AChRs, we studied effects of innervation on neuronal glutamate receptors. Motor neurons grown together with interneurons were six times more sensitive to glutamate than were motor neurons grown alone. Interneurons also altered the distribution of glutamate receptors. Sharp peaks of sensitivity were found along motor neuron dendrites when they were grown together with interneurons. The parallel with regulation of muscle AChRs by synaptic inputs is striking. To my knowledge, no effort has yet been made to isolate potential inducing factors.

Another interesting parallel with neuron muscle interaction is the observation that more than 25 percent of the motor neurons were dye coupled to nearby interneurons during early stages of synapse formation. Direct coupling was not detected after the first week in vitro.

Richard O'Brien led the spinal cord studies. I met O'Brien at Harvard where he was a medical student. He completed his thesis research in St. Louis before returning to Harvard and the Massachusetts General Hospital. In addition to his own work, Rich improved the work of other students in my lab, students in other labs, and many of the faculty. He is a gifted, inspiring physician and scientist.

During this time at Washington University, Larry Trussell conducted a remarkable series of experiments on rapid desensitization of glutamate receptors. He found that desensitization was fast enough to terminate synaptic currents. Thus receptor desensitization must be considered along with diffusion and reuptake to be a mechanism for terminating glutamate action. He also found that steady-state desensitization occurred at concentrations far below those needed for receptor activation, implying that spontaneous release of glutamate at or near synapses may regulate the efficacy of glutamatergic synaptic transmission.

Harvard Redux (1990–1998)

In 1990, important projects in my lab were in transition, and our youngest children went off to college. Therefore, when an extraordinary offer came from Harvard, we considered it carefully and, once again stepped into the unknown. Ruth was sad to leave Boston in 1981, but she was even sadder to leave St. Louis. She had thrived in the Department of Psychiatry completing her PhD thesis (from Boston University) and a master's degree in psychiatric epidemiology, initiating important projects in medical sociology and neuroethics, and taking on important teaching roles.

As noted earlier, the Harvard Department of Neurobiology was known for its cloistered environment. No joint appointments were made, and they brought only one scientist forward for promotion to tenure in the previous 26 years. In the next nine years, we recruited nine investigators to the Quadrangle Department. Eight were promoted to tenure (Wade Regher, Linda Buck, Rod MacKinnon, Chuck Weitz, Clay Reid, Gary Yellen, John Assad, and Rick Born). Two, Linda Buck and Rod MacKinnon, went on to win Nobel Prizes. Elio Raviola and David Paul were invited to join the Neurobiology Department when the Anatomy Department was transformed into a Cell Biology Department under the leadership of Marc Kirschner. In addition, we offered appointments to scholars in hospital-based departments, including Mike Greenberg, David Clapham, Clifford Woolf, Richard Masland, David Corey, and Steve Hyman. The sense of community was further enhanced when we abolished the Neurobiology Graduate Program and took a leadership role in the school-wide Neuroscience Program.

My labs at the Quadrangle were not ready in 1990 so my first scientific home on returning to Boston was located in Charlestown in the MGH research building. I was asked to chair the MGH Executive Committee on Research so I got a broad overview of this remarkable institution, including its affiliate MacLean Hospital. The plain fact is that the hospital's neuroscience community was more diverse than Harvard's Neurobiology Department. But my love for the Quadrangle community was strong and that just added to my motivation.

I want to single out two recruits. Linda Buck wanted to exploit her remarkable discoveries made with Richard Axel regarding the mega-family of olfactory receptors. It was a difficult time for her with a small lab, but she advanced stride for stride with the larger, talented, well-funded Axel group. And she succeeded brilliantly. She received as much financial and emotional support as I could provide. Chuck Weitz, rather than pursue his successful postdoctoral work with Jeremy Nathans on photopigments, switched fields to study the molecular workings of circadian clocks. He wrote excellent grants, but none were funded despite excellent reviews. He followed his interests and did not give up and now is a leader in this rapidly evolving field. Our ability to support him through such difficult times might not be possible to duplicate in the current funding environment.

These were wonderful years at Harvard. We worked together, argued together, and laughed together. Jon Cohen and Bruce Bean stand out in my mind. Both focused on ion channels, Bean via electrophysiology; Cohen via biochemistry and molecular pharmacology. Both are role models of what an academic scientist should aspire to. Cohen has a high quotient of common sense that I found invaluable in my attempts to manage science in the department and in my own lab. Scores of neuroscience graduate students owe him a great debt of gratitude.

A rather unique effort during these years was the establishment of the Mind, Brain and Behavior (MBB) Initiative. Neil Rudenstein, the president of the university, called for proposals that would bring together scholars from departments throughout the university. Steve Hyman was involved from the start. MBB began as a discussion forum involving members of various departments, including psychology, biology, and English, as well as members of the business school and medical school. Our hope was that it eventually would include interdisciplinary research, but financial pressures stalled that effort. Within one year, MBB became the second largest concentration for Harvard undergraduates. The name and the interdisciplinary philosophy of the Harvard MBB Initiative were adopted some years later by Columbia University for their ambitious neuroscience project in Manhattanville.

Further Studies of NRG/ARIA at the nmj and in the CNS

Several experiments conducted at this time provide additional evidence for roles for NRG/ARIA at the nmj and in the CNS. We found that NRG immunoreactivity and mRNA are present in embryonic motor neurons and the protein is concentrated at nmjs soon after the arrival of motor axons. Jeff Loeb found that NRG accumulated along motor axons and in the synaptic cleft in association with heparan sulfate proteoglycans. NRG epitopes, identified by immuno-gold EM are associated with the presynaptic side of the cleft basal lamina as expected of a factor released from the nerve terminal.

In collaboration with Cohen, we showed that the NRG receptors erB2 and erB4 were located in the depths of the secondary postsynaptic folds at the adult rat nmj. Gabriel Corfas found that NRG increased the number of voltage-gated Na channels in myotubes adding to our notion that this trophic factor increased the safety factor for neuromuscular synaptic transmission.

Al Sandrock led a group in my lab that prepared and studied NRG heterozygous mice and showed that the NRG-deficient mice were myasthenic. This was brought out during repetitive stimulation in the presence of low doses of curare. The AChR density at motor endplates, as judged by mepp amplitude and BTX binding, was reduced in the heterozygotes.

Despite this evidence for the importance of NRG, it seemed likely in 1993 and even more so in 2016, that NRG was probably not the sole factor influencing the accumulation of AChRs at developing nmjs. As expected of complex biologic phenomena, other inducing substances may serve the same or supplementary functions. AGRIN, identified by Jack McMahan in the early 1980s based on its ability to promote aggregation of muscle AChRs, was a likely candidate. Steve Burden has provided strong evidence that AGRIN, acting through its recently discovered receptor Lrp4 and the muscle specific kinase (MuSK) are important regulators of AChR accumulation at developing nmjs.

NRG is expressed in cholinergic neurons throughout the rat brain, including cranial motor nuclei and the nucleus basalis. It is not restricted to cholinergic neurons. Nerve terminals on spinal motor neurons are intensely labeled. NRG is expressed throughout DRG, but the α1NRG isoform is present only in the smallest neurons and only in the most superficial lamina in the spinal cord. Perhaps this molecule is involved in the regulation of pain at the spinal cord level. NRG is abundant in the molecular layer of the cerebellum. Bomie Han found that NRG is processed and released from subcellular fractions containing synaptosomes.

NRG also may be involved early in neuronal development. Cells in the germinal (subventricular) zones of the embryonic and early postnatal cerebellum and telencephalon were heavily labeled, suggesting roles for NRG in the proliferation or migration of neuronal and glial precursors. Interest in central actions of NRG has grown over the years. Members of the NRG and erbB families have been implicated as risk genes in autism, schizophrenia, and other neuropsychiatric disorders.

Tim Vartanian, a neurologist interested in multiple sclerosis, joined my lab at this time. We found that NRG promoted the proliferation of oligodendrocyte/astrocyte precursors (O2A cells) via actions on erB2 and erB4 receptors. NRG also promoted the proliferation of Schwann cells, but in this case, the effect was mediated via action on erB2 and erB3 receptors. This work added to the growing interest in neuron–/glia trophic interactions and demyelinating disorders.

The Salk Institute

I was invited to become a nonresident fellow of the Salk Institute in the late 1980s, and I remained in that role for more than 12 years. My first contacts with the Salk Institute began in the early 1970s when I became aware of the work of Steve Heinemann, Jim Patrick, Dave Shubert, Joe Henry Steinbach, and their colleagues on transformed muscle and neuronal cell lines. Subsequently, I followed Heinemann's work in the molecular coning and characterization of nicotinic AChR receptor and glutamate receptor subunits. Like the MBL, the Salk Institute became a major influence on my development as a scientist and as a citizen of the larger community of life scientists.

Nonresident fellows attend faculty meetings and research retreats. They are encouraged to spend significant periods of time on campus at any time of the year. They contribute to the evaluation of candidates for promotion to tenure, they mentor junior faculty, and their comments are sought on major new research programs at the Salk Institute. In other words, it is a serious commitment. But it was always a rewarding experience as well, and over the years, a remarkable group of Young Turks on the faculty evolved into an even more remarkable set of Middle-Age Turks who assumed a great deal of responsibility in building and governing the Salk Institute. Inder Verma, Ron Evans, Wylie Vale, Terry Sejnowski, Joanne Chory, Chuck Stevens, Tom Albright, Rusty Gage, and Heinemann, among others, became world leaders in their fields. The next generation of Salk neuroscientists, too numerous to list here, is extremely strong.

Many Salk scientists lived on the edge without the advantage of a large Salk endowment. They received many attractive offers to move, but few left. I can think of three reasons for this seemingly irrational behavior. First is the physical beauty of the Louis Kahn–designed campus. The main patio grooved along its entire length by a flowing canal that seems to cascade off the edge of the earth into the Pacific Ocean and beyond suggests the limitless possibilities of fundamental science. Second is the strong sense of community. The layout of the labs contributes to this coming together, but mutual respect and years of collaboration and friendship is unmatched in my experience. Third is the growth of the surrounding biomedical community led by the closer ties to the neighboring University of California–San Diego (UCSD). New MRI technologies and stem cell research facilities, developed in collaboration with UCSD, are important added resources for Salk scientists.

NIH Redux (1998–2001)

When Harold Varmus, then director of the NIH, offered me the position of director of the NINDS, it made me rethink my priorities once again. Since

my first experience at the NIH in 1966, I have had a high regard for the institution. Our universities are the envy of biomedical scientists the world over largely because of NIH-funded research. In 1953, when Ovita Culp Hobby dedicated the Clinical Center at the NIH, the NIH budget was $12 million. As of 2016, it is more than $30 billion. The impact of an active NIH director in influencing the course of science is difficult to overestimate.

I had addressed national and international audiences before, but the bully pulpit of an NIH director backed by a $1 billion plus budget is on another level. I traveled widely in this country, Europe, England, China, and Japan. Everyone I met wanted to emulate the NIH policy of funding science based on peer review rather than local politics.

Ruth was attracted to the NIH as well. Always the idealist, she saw the need to consider ethical issues on a national scale. Also, happy memories of our first tour at the NIH and our lifelong friends in Bethesda were important considerations.

Colleagues in Boston questioned my judgment in leaving the extraordinary faculty and students at Harvard to work with civil servants, demanding patient advocates, and self-centered members of Congress. But I found dedicated, talented scientists among the NINDS. The passion, knowledge, and firsthand experiences of advocacy groups were inspirational. Varmus had assembled a strong team of NIH directors and spirits were high as the NIH was in the middle of a five-year doubling of its budget.

Within NINDS, I worked with extraordinary people. The talented chief operating officer, Kevin Kirby, coordinated the efforts of Story Landis, director of intramural research; Connie Atwell, director of extramural research; and Mary Meirs, director of science policy and analysis; and he helped guide our large science staff. He also helped junior and senior officers achieve their full potential. We abolished long-standing fiefdoms within the NINDS and delegated more authority to junior staff members. Spirits soared and so did productivity.

Parkinson's disease was a major focus. At the request of Congress, I led several other Institutes in composing a blue sky Parkinson's disease plan that called for a $1 billion budget. L-DOPA, introduced in 1957, is a "miracle" drug that can reverse or reduce the three cardinal symptoms of Parkinson's disease, but the beneficial effects wear off in time, and debilitating side effects, including dyskinesias, come to the fore.

Deep brain stimulation (DBS) of neurons within the basal ganglia had been successful in selected cases, and the first results called for more intensive efforts. Mahlon DeLong, who became involved in NINDS programs during my tenure as director, shared the 2014 Lasker Award for his brilliant and brave work with nonhuman primates on the physiology of the basal ganglia and their roles in movement disorders. He identified the subthalamic nucleus as a key point of intervention. DeLong shared the Lasker Award with Amin Benabid who refined surgical and stimulation protocols so that they now benefit hundreds of thousands of patients.

In my first encounter with the Senate Appropriations Committee, I was put on the spot by the chair Arlen Spector when he asked when I thought we would cure Parkinson's disease. I hesitated citing recent advances but also explained that future advances were difficult to predict. He did not relent in his demands to know if it would take 5 or 10 years. I said, "within 10 years—I hope." I have not yet lived that down and can still hear the gasps of my fellow NIH directors. My reasoning depends on the word "cure." DBS is not a cure in that the degenerating dopaminergic neurons are not restored or replaced, but it is a cure in that normal function can be restored and maintained, in some cases, for more than 15 years. Not bad for a neurodegenerative disorder.

Working together with extramuaral and intramural scientists and with strong advocacy groups, the staff organized a series of workshops that changed the course of research in several areas. One effort that stands out in my mind was a White House–supported conference on "Curing Epilepsy: Focus on the Future." The emphasis of this meeting, which took place in 2000, was on mechanisms of epileptogenesis and their reversal rather than on symptomatic treatments. Benchmarks were established that have been reviewed periodically over the past 15 years. Cures have not yet been achieved, but a feeling of optimism persists.

Other major efforts during my stay at NINDS included the following: revision and expansion of our clinical trials program; a new effort in high-throughput screening for therapeutics to treat rare "orphan" diseases; a renewed emphasis on multiple sclerosis; supporting emergency room stroke centers to facilitate rapid diagnosis and treatment; an expanded program to improve treatment of spinal cord and brain injury; numerous presentations before Congress; and improved communication about neurological disorders, working with Marion Emr to create a revitalized go-to website.

We encouraged collaborations via Program Project Grants (R01) and U54 contracts. But the emphasis remained on individual investigator R01 grants. About 80 percent of our grants each cycle were awarded based strictly on review panel recommendations. That left 20 percent to be selected by the staff, sometimes out of order, based on the originality and promise of the work and the needs of the field. This is a responsibility we all accepted.

Perhaps my most enduring legacy at the NIH is the role I played in creation of the John Porter Neuroscience Center. I understand the special interests that convinced Congress to create separate Institutes, but a case can be made that the science underlying psychiatric and neurological disorders, substance abuse, communicative disorders, aging, and vision is coming closer together. Scientists funded by the separate Institutes have a lot to learn from each other. It made sense to set an example for the nation by bringing labs in different Institutes together in one building. This dream was initiated with Steve Hyman and Bob Desimone from NIMH and Story Landis in NINDS. The magnificent 600,000-square-foot building is now a

reality on the NIH campus. It is a fitting tribute to John Porter, congressman from Illinois, who helped shape the NIH budget during the doubling years.

Continued Laboratory Research

Experiments on neuronal nicotinic AChRs continued at the NIH with Y. Liu and Q. Chang. Chronic application of NRG increased the number of α7-nAChRs in hippocampal pyramidal neurons and on GABAergic interneurons. In addition to this effect on neuronal receptors, we found that NRG increased the proliferation of neuronal precursors isolated from E11 mouse telencephalon. Mary Anne Mann, who moved with me from Harvard, managed the lab and continued our experiments on nerve-muscle synapses. I enjoyed walking from my office in Building 31 up the hill to my lab in Building 36 where I worked so many years ago.

Dark Clouds Form

In 2001, after a period of five years in which the NIH budget was doubled, dark clouds gathered on the horizon that are still very much with us. Budgets were flat or cut, and the purchasing power in the research market plummeted. Earmarks from Congress kept coming, but with no added money. Such requests are important in that they serve to focus attention, but without added funds, other disorders and basic research are further squeezed. I also worry about the length of time it takes a young scientist to achieve independence. On average, the first independent NIH grant is received at age 42. I was a tenured professor at that age and the father of four children.

My departure from the NIH was unexpected but it was inevitable. When Harold Varmus announced his retirement, Donna Shalala, secretary of U.S. Department of Health, Education, and Welfare, nominated me to replace him as the NIH director. Shalala was informed about the NIH and was sensitive to the political climate in Washington. She was also bold. Realizing that any nomination was at risk in the final months of the Clinton administration, she decided it was worth the effort. After weeks of positive interviews on the Hill, and meetings with FBI investigators, the relevant senate committee was reluctant to confirm my nomination.

My advocacy for human embryonic stem cell research may have been an unstated factor. The Dickey/Wicker amendment to the U.S. Department of Health and Human Services (HHS) appropriations bill, adopted in 1996, prohibited the creation of stem cell lines from human embryos using NIH funds. My strong statements in favor of human stem cell research would have been appreciated during my first tour at the NIH in 1970, but we were in a different era in 2001, and I was at risk. When it became clear that politics might prevail, the secretary, reluctantly, decided that the best path

forward for the administration (and for me) was to withdraw the nomination. Lawyers inside and outside of HHS advised me to consider leaving government service.

Columbia University (2001–2006)

My decision was made easier when Columbia offered an interesting alternative. George Rupp, then president of Columbia University and Jonathan Cole, provost, recruited me as executive vice president (EVP) for Health Sciences and dean of the Medical School. This is a big job at Columbia, as the deans of four schools (Dental, Medicine, Public Health, and Nursing) and the College of Physicians and Surgeons (P&S) report to the EVP. Despite this, I asked that my title be expanded to EVP for Health and Biomedical Sciences. Significant challenges included a large structural deficit and a severely balkanized medical center with 384 independent business units. This Tower of Babel needed a coherent sense of community beyond their own microenvironments. Rupp and Cole were enormously helpful as I was forced to learn on the fly.

Once again, Ruth gave up a rewarding position to begin anew in a different context. She helped in many ways over the next five years especially as a strong partner in many fundraising ventures. She also established a university-wide Center for Bioethics, contributed to training programs, and established new courses for medical and graduate students. Her Ethics for Lunch program organized with Ken Prager and supported by the Arnold P. Gold Foundation remains an important forum for discussion of difficult ethical issues that arise during the course of hospital practice.

Our move to Manhattan completed an odyssey from New York City to Seattle to Bethesda, to Boston, to St. Louis, back to Boston, back to Bethesda, and, finally back to New York City. I assembled a strong administrative staff that included Kevin Kirby (COO), who joined me from the NIH; Michael O'Conner (CFO); Harvey Colten (academic affairs); Joe Tennenbaum and Eric Rose (clinical affairs); Marian Carlson (research affairs); Marian Jakubiak (executive assistant); and Marion Greenup (operations officer).

We embarked on a planning process that addressed research, education, and patient care in each of the four schools. In collaboration with the New York–Presbyterian Hospital, we also conceived a long-range space plan for growth of the Medical Center. More than 200 faculty, students, and administrators were involved. When we began, spirits were low as the NIH budget continued to fall, and the horror of the September 11 terrorist attacks that occurred in plain view from Washington Heights became a chronic worry.

Perhaps our most significant accomplishment was a change in spirit throughout the Medical Center. The atmosphere was more open, hopes were raised, and enthusiasm was high. I believe that the Medical Center was on a better tack when I stepped down in 2006. Some specific accomplishments follow.

The Herbert Irving Cancer Center was completed and a new director, Ricardo Della Favera, was appointed. The Human Genome Center was revitalized with the recruitment of James Rothman, a future Nobel Laureate, as director. A new Center for Computational Biology was created under the leadership of Andreas Califano and Barry Honig. The sophisticated mathematics and statistics promoted by this center hold the keys to a better understanding of the human genome and the future of therapeutics. A new Motor Neuron Research Center was established, and the renowned Center for Neurobiology and Behavior was transformed into a Department of Neuroscience to give the program a "place at the table" of medical center affairs. A new dean for Dental Medicine was appointed, and, at the medical school, new chairs were recruited in genetics, physiology, psychiatry, ob/gyn, medicine, and anesthesiology. Interim chairs were appointed in biochemistry, microbiology, radiation, oncology, and pediatrics.

Nearly all of the endowed chairs at Columbia Medical Center had been donated by grateful patients and resided in clinical departments. Over the course of five years we established many chairs in preclinical departments. P&S alumni are among the most generous in the university. A Health Science Council was established to raise awareness and help raise additional funds. Roy Vagelos, a P&S alumnus, was the first director of the council. Our goal was raise $1 billion, and we came close by the time I stepped down.

At the request of the New York Police Department, Fred Kass, now vice chair of psychiatry, and Dr. Ellen Stevenson organized a remarkable effort to treat thousands of New York City policemen and firemen who suffered from post-traumatic stress disorder following September 11. Confidentiality was essential to maintain job security and avoid stigma. This remarkable, still underappreciated, effort is a wonderful example of how talented university faculty can help their community.

The faculty practice plan is a $500 million-plus per year business, but many physicians were dissatisfied over issues ranging from electronic medical records, billing procedures, physical resources, compensation, and governance. This income adds significantly to the borrowing capacity of the university so the trustees were rightly concerned. We radically changed the plan, now called Columbia Doctors, granting greater autonomy to the physicians. This overhaul took two years to accomplish as visits were made to medical centers across the country and an executive director of the plan was recruited. Physicians tell me that this transformation will be my greatest legacy at Columbia.

A significant effort was devoted to revamping the medical school curriculum with an emphasis on tools for self-learning. The Glenda Garvey Teaching Academy was established to enhance the teaching mission throughout the health sciences, with divisions in each of the four schools at the medical campus.

Renovation of the library was begun. Discussion of a new student education center was given impetus by a pledge from Roy and Diana Vagelos. We did not accomplish all of our goals. A center for outpatient medicine and a hotel for families to be constructed on the 165th Street parking lot remained a dream. A new neuroscience research building at the Medical Center was put aside as plans for the university-wide MBB Initiative evolved.

With the merger of the New York Hospital and Presbyterian Hospital, the name Columbia Presbyterian Medical Center no longer existed. Something had to change. After a great deal of consultation, we decided on a new name, the Columbia University Medical Center. Objections were raised. Many distinguished faculty bore the Columbia Presbyterian Medical Center name proudly. The schools of nursing and public health felt that their unique missions were not sufficiently recognized. Despite the challenges and distractions, the new brand gradually took hold. Marilyn Laurie, a trustee with expertise in rebranding efforts, was particularly helpful.

The New York–Presbyterian Hospital presented many benefits and many challenges. Our interests were aligned, but they were not always identical. When one hospital is dealing with two academic centers, there are bound to be tensions and disagreements. We reached agreement on appointment of clinical department chairs and many other matters, including insurance claims and academic programs of mutual interest. We struggled to maintain hospital affiliations that were steeped in history and social awareness, including Harlem Hospital, Mary Imogene Bassett, and St. Lukes–Roosevelt. Business decisions often carried the day.

The university also had interests that were aligned with the Medical Center but were not always identical. When money is in short supply, tensions emerge. When I arrived, Rupp and the trustees were considering a major expansion of the campus. Two options were discussed: a large area between 125th and 133rd Street, Broadway, and the Hudson River versus a comparable area downtown, farther away from the Medical Center. In my role as EVP for Biomedical as well as Health Sciences, I had spent significant time trying to integrate neurosciences, psychology, molecular biology, and electrical engineering across the campuses at Morningside Heights and Washington Heights, so I advocated strongly for the former. Manhattanville, as the 125th Street option came to be called, is a grand vision now being realized by President Lee Bollinger and his team. The lead project in Manhattanville is a new building to house the MBB Initiative headed by Tom Jessell.

There are only 24 members of the Columbia University Board of Trustees, most of whom focus on the Morningside Heights campus. The small Health Sciences Trustee Sub-Committee knew little about the inner workings of the medical campus in Washington Heights, but they were eager to learn. We initiated regular meetings uptown, including tours of the physical plant, and I offered a series of Medical School 101 tutorials. They

were shocked to realize how little the university contributed to salaries or research funding for even the most distinguished medical scientists. This, of course, is not unique to Columbia. As the trustees learned more, their enthusiasm grew.

When I accepted the EVP position, I believed that this individual, like a lab chief, or a department chair, or even a NIH director, could be a role model for faculty and students of every stripe. I now understand Samuel Johnson's sentiments expressed in the preface to the first edition of his *Dictionary of the English Language* (1979).

> It is the fate of those who toil at the lower employments of life — to be exposed to sensure [*sic*], without hope of praise; where success would have been without applause and diligence without reward. — Among these unhappy mortals is the writer of dictionaries [EVP for Health Sciences] — . Every other author may aspire to praise; the lexicographer [EVP] can only hope to escape reproach — .

The dictionary worked out well for Johnson, and the Columbia EVP job worked out well for me. I am proud of our accomplishments during a difficult time in academic medicine.

My lab remained active throughout this hectic period, but with much less input from me. Marian Mann, a former student at Harvard, who moved with me to the NIH, and then to Columbia, carried the ball as Senior Research Assistant. We collaborated with Bob Darnell at Rockefeller University who found that AGRIN was regulated by the transcription factors NOVA1 and NOVA2. We found that when AGRIN was restored in NOVA1, 2 knockouts, synaptic AChR clusters reappear, but the mice remain paralyzed. A proximal deficit, perhaps on motor neuron excitability, may be at work. This apparently simple system holds many lessons for more complicated multifactorial brain disorders. We also continued work during these busy years on effects of NRG on CNS neurons.

Simons Foundation (2006–present)

Jim and Marilyn Simons established the Simons Foundation in 1993 with the goal of supporting research in basic science and mathematics. With time, they became interested in research on autism and began by awarding a small number of grants. They picked excellent investigators, but as they began to explore the complexity of autism science, they sought advice. Several informal conversations and an important roundtable meeting devoted to autism research followed.

Soon after I stepped down as dean and began a sabbatical year, Jim Simons and I shared pastrami sandwiches at Arties' delicatessen on Broadway. He planned to ask me to help search for a director of the expanding

Simons Foundation autism research program. Instead, he offered me the job. I accepted immediately. This impulse has led to one of the most challenging experiences of my career in science and to an enduring friendship.

My interest in autism and other developmental disorders had been awakened at the NIH. It was a frustrating time there because, despite the efforts by informed advocacy groups such as Cure Autism Now and the National Alliance for Autism Research, little progress had been made. Two things were clear at the start. First, autism is primarily a genetic disorder so a search for genetic variants that enhance the risk of autism offered the best route to a deeper understanding of autism traits. Second, the field needed an influx of excellent scientists to explore molecular, cellular, and systems mechanisms suggested by genetic discoveries.

Michael Wigler, a long-standing colleague of Jim's, had already decided to study autism. His insight, perhaps influenced by his studies of cancer genetics, led to a search for de novo genetic variants in autistic individuals. Such variants, which arise in the germ line of a parent, are highly penetrant. Each one is extremely rare (less than 1 percent of cases), but together they may contribute to a large fraction of individuals on the autism spectrum. Therefore, a large sample of "simplex" families was needed. This is easier said than done. We established the Simons Foundation Autism Research Initiative (SFARI) to coordinate the efforts of 12 university autism clinics across the United States and Canada to recruit nearly 3,000 simplex families that include one and only one individual on the autism spectrum.

Led by Cathy Lord, clinicians in the clinics and staff at the foundation standardized clinical evaluations, collected blood samples, and shared data. The result is a cohort that set new standards for the field of neuropsychiatric disorders and that will be used in autism research for years to come. We showed that complex behavioral assessments could be quantitated and reproduced from site to site.

At the time of writing, more than 70 de novo genetic risk factors have been identified with high confidence. Current estimates are that more than 300 genes contribute to the risk of autism. This is a big number, but we must remember that we are dealing with human social cognition, communication, and persistent behaviors. Efforts to identify more de novo variants and to extend the genetic landscape beyond de novo mutations are under way.

Gene discovery will provide important clues, but the human brain lies between genes and behavior. We must move beyond current oversimplifications, such as autism is a "synaptic disorder" or an "excitation/inhibition imbalance" or a "disconnection syndrome," and confront the complexity of coding and dynamics in neuronal populations involved in social cognition.

Perhaps our greatest accomplishment in the first years was the recruitment and shaping of an interactive community of superb scientists from diverse fields to focus on autism. Our meetings at the foundation are exciting, informative, and tinged with a sense of urgency. Many participants

feel that the Simons Foundation has revolutionized the study of autism. What did it take? Money helps, but I believe the community would not have coalesced if scientific opportunities were not evident. Powerful cell and molecular and imaging tools offer new approaches to pressing questions. Vision and leadership are also essential, and they can be practiced at the foundation with few constraints. I cannot overemphasize the impact of Jim and Marilyn Simons. They "show up" and participate on a meaningful level at staff meetings, seminars, journal clubs, and major lectures. Their curiosity, generosity, values, and passion inspire internal staff and external supported scientists. In many ways, I am reliving my role as director of the NINDS during the era when Congress recognized the importance of science and provided adequate funds.

The notion of a "social brain" has emerged as a useful concept in autism studies. The definition has become more specific in recent years. This is a functional rather than an anatomical designation. My bet is that neural correlates of autism will be found in these interconnected regions. I also believe that we must look for key changes in brain function at or before birth. Even though the seeds of autism are sown early, studies of mice bearing mutated human genes have shown that abnormal behaviors can be reversed in adult life. It is never too late, and we must never lose hope for improvements in the quality of life for those on the autism spectrum.

The Simons Foundation has grown from fewer than 10 employees when I began in 2006 to more than 120 just 10 years later. We moved from adequate space (about 12,000 square feet) to magnificent quarters (about 75,000 square feet) a few blocks away in Chelsea. The scope of the foundation has expanded to include many new topics in life sciences, mathematics, and physics. I stepped down as director of SFARI in 2013, to assume the role of chief scientist. I have remained involved in SFARI, but my charge in this new role is to foment, advise, and help decide on new projects.

Several large collaborations named "Big Ideas" projects have emerged in recent years. Each one addresses an important topic and each one must promise to revolutionize thinking in their field; they must have enlisted excellent scientists; they must define landmarks and demonstrate progress over the course of the grant; and they must demonstrate a need for funding as well as a plan for continued funding after 5–10 years. Such collaborations emerge from extensive internal discussions as well as discussions with candidates and with outside experts. The Simons Collaboration on Data Analysis, headed by Leslie Greengard, is the first in-house Big Idea project.

It has been exciting to work with the Simons Senior Science Staff: Marian Carlson in life sciences, Yuri Tschinkel and Andy Millis in mathematics and physics, and Leslie in planning and evaluating new proposals. A sample of collaborations now under way includes microwave evidence for the Big Bang, algebraic geometry, the many electron problem, ocean microbiota, the origin of life, and the global brain. In addition, the foundation has

provided support for the New York Genome Center and for Cryo-Electron Microscopy at the New York Structural Biology Center. It often appears to me that Jim Simon uses the foundation as his laboratory to learn more about the world we live in and beyond. I often think of the Simons Foundation as a young Howard Hughes Medical Institute, but with a broader mandate. It is a joy to help shape the journey.

At the moment, I am most involved with the Simons Collaboration on the Global Brain (SCGB), an effort to identify principles of coding and dynamics in populations of neurons that are active in the formation and recall of memories, motor planning, spatial navigation, other decisions, and other internal mental states interposed between sensation and motor output. Experimental approaches require electrical or optical recordings at single-cell resolution in real time. Novel statistical approaches are needed to detect neural signatures that may not be evident in the original recordings.

This ambitious, transformative plan first suggested by Bill Newsome and now directed by David Tank is advised by a steering committee that includes Tony Movshon, Larry Abbott, Adrienne Fairhall, Dora Angelaki, Newsome, and me. We have awarded 41 grants—each one involving collaboration between experimentalists and mathematicians. The challenge now is to promote meaningful interactions among the various groups in the SCGB. Curiosity drives most investigators in the SCGB. Quite simply, they seek a better understanding of mental life. I share that respect for curiosity-driven research, but I do hope that such approaches will lead to useful biomarkers for neuropsychiatric disorders.

In Sum

In my career, I have worked my way up from the nmj to the spinal cord to the midbrain, to the basal ganglia to the archicortex, to the neocortex, and now to the social brain, cognition, and consciousness. In retrospect, this was my dream from the start as I entered medical school in 1960. Each step along the way demanded intense study of the relevant literature and experimental approaches. Each step has had its unique rewards. As the director of technology transfer at MIT told me years ago, "Gerry, at your age it is a privilege to have a steep learning curve."

Throughout my career, I have tried to pick important questions, find important collaborators, persevere, and have fun. The profound questions have not changed significantly over the decades, but new techniques have revolutionized our approaches to these persistent questions.

Beyond my own laboratory, I believe that I have influenced the productivity and, ultimately, the careers of many scientists, young and old.

What is next? I look forward to less social and academic noise. I would like more time to write to clarify my thoughts about the past and about future directions. As I think back over my time in research, I am satisfied

but not completely fulfilled. I accept the fact that I am no longer a postdoctoral fellow at the NIH, but thoughts about unfinished experiments recur and exciting new ideas emerge. As time slips away, Ruth and I want to spend more time with our wonderful children, grandchildren, extended family, and close friends.

Selected Bibliograpy

Berg, D.K., and G.D. Fischbach. 1978. Enrichment of spinal cord cell cultures with motoneurons. *J Cell Biol*. 77:83–98.

Buc-Caron, M.H., P. Nystrom, and G.D. Fischbach. 1983. Induction of acetylcholine receptor synthesis and aggregation: partial purification of low-molecular-weight activity. *Dev Biol*. 95:378–386.

Buonanno, A., and G.D. Fischbach. 2001. Neuregulin and ErbB receptor signaling pathways in the nervous system. *Curr Opin Neurobiol*. 11:287–296.

Bursztajn, S., and G.D. Fischbach. 1984. Evidence that coated vesicles transport acetylcholine receptors to the surface membrane of chick myotubes. *J Cell Biol*. 98:498–506.

Carr, C., G.D. Fischbach, and J.B. Cohen. 1989. A novel 87,000-Mr protein associated with acetylcholine receptors in *Torpedo* electric organ and vertebrate skeletal muscle. *J Cell Biol*. 109:1753–1764.

Chalazonitis, A., and G.D. Fischbach. 1980. Elevated potassium induces morphological differentiation of dorsal root ganglionic neurons in dissociated cell culture. *Dev Biol*. 78:173–183.

Chang, Q., and G.D. Fischbach. 2006. An acute effect of neuregulin 1 beta to suppress alpha7-containing nicotinic acetylcholine receptors in hippocampal interneurons. *J Neurosci*. 26:11295–11303.

Choi, D.W., D.H. Farb, and G.D. Fischbach. 1977. Chlordiazepoxide selectively augments GABA action in spinal cord cell cultures. *Nature*. 269:342–344.

Choi, D.W., D.H. Farb, and G.D. Fischbach. 1981a. Chlordiazepoxide selectively potentiates GABA conductance of spinal cord and sensory neurons in cell culture. *J Neurophysiol*. 45:621–631.

Choi, D.W., D.H. Farb, and G.D. Fischbach. 1981b. GABA-mediated synaptic potentials in chick spinal cord and sensory neurons. *J Neurophysiol*. 45:632–643.

Choi, D.W., and G.D. Fischbach. 1981. GABA conductance of chick spinal cord and dorsal root ganglion neurons in cell culture. *J Neurophysiol*. 45:605–620.

Cohen, S.A., and G.D. Fischbach. 1973. Regulation of muscle acetylcholine sensitivity by muscle activity in cell culture. *Science*. 181:76–78.

Cohen, S.A., and G.D. Fischbach. 1977. Clusters of acetylcholine receptors located at identified nerve-muscle synapses in vitro. *Dev Biol*. 59:24–38.

Connolly, J.A., P.A. St John, and G.D. Fischbach. 1982. Extracts of electric lobe and electric organ from *Torpedo californica* increase the total number as well as the number of aggregates of chick myotube acetylcholine receptors. *J Neurosci*. 2:1207–1213.

Corfas, G., D.L. Falls, and G.D. Fischbach. 1993. ARIA, a protein that stimulates acetylcholine receptor synthesis, also induces tyrosine phosphorylation

of a 185-kDa muscle transmembrane protein. *Proc Natl Acad Sci U S A*. 90:1624–1628.

Corfas, G., and G.D. Fischbach. 1993. The number of Na+ channels in cultured chick muscle is increased by ARIA, an acetylcholine receptor-inducing activity. *J Neurosci*. 13:2118–2125.

Corfas, G., K.M. Rosen, H. Aratake, R. Krauss, and G.D. Fischbach. 1995. Differential expression of ARIA isoforms in the rat brain. *Neuron*. 14:103–115.

Dichter, M.A., and G.D. Fischbach. 1977. The action potential of chick dorsal root ganglion neurones maintained in cell culture. *J Physiol*. 267:281–298.

Dubinsky, J.M., and G.D. Fischbach. 1990. A role for cAMP in the development of functional neuromuscular transmission. *J Neurobiol*. 21:414–426.

Dubinsky, J.M., D.J. Loftus, G.D. Fischbach, and E.L. Elson. 1989. Formation of acetylcholine receptor clusters in chick myotubes: migration or new insertion? *J Cell Biol*. 109:1733–1743.

Dubinsky, J.M., M. Morgan, and G.D. Fischbach. 1988. Variation among acetylcholine receptor clusters induced by ciliary ganglion neurons in vitro. *Dev Biol*. 130:209–219.

Dunlap, K., and G.D. Fischbach. 1978. Neurotransmitters decrease the calcium component of sensory neurone action potentials. *Nature*. 276:837–839.

Dunlap, K., and G.D. Fischbach. 1981. Neurotransmitters decrease the calcium conductance activated by depolarization of embryonic chick sensory neurones. *J Physiol*. 317:519–535.

Engisch, K.L., and G.D. Fischbach. 1990. The development of ACH- and GABA-activated currents in normal and target-deprived embryonic chick ciliary ganglia. *Dev Biol*. 139:417–426.

Engisch, K.L., and G.D. Fischbach. 1992. The development of ACh- and GABA-activated currents in embryonic chick ciliary ganglion neurons in the absence of innervation in vivo. *J Neurosci*. 12:1115–1125.

Falls, D.L., D.A. Harris, F.A. Johnson, M.M. Morgan, G. Corfas, and G.D. Fischbach. 1990. Mr 42,000 ARIA: a protein that may regulate the accumulation of acetylcholine receptors at developing chick neuromuscular junctions. *Cold Spring Harb Symp Quant Biol*. 55:397–406.

Falls, D.L., K.M. Rosen, G. Corfas, W.S. Lane, and G.D. Fischbach. 1993. ARIA, a protein that stimulates acetylcholine receptor synthesis, is a member of the neu ligand family. *Cell*. 72:801–815.

Farb, D.H., D.K. Berg, and G.D. Fischbach. 1979. Uptake and release of [3H]gamma-aminobutyric acid by embryonic spinal cord neurons in dissociated cell culture. *J Cell Biol*. 80:651–661.

Fenelon, K., J. Mukai, B. Xu, P.K. Hsu, L.J. Drew, M. Karayiorgou, G.D. Fischbach, A.B. MacDermott, and J.A. Gogos. 2011. Deficiency of Dgcr8, a gene disrupted by the 22q11.2 microdeletion, results in altered short-term plasticity in the prefrontal cortex. *Proc Natl Acad Sci U S A*. 108:4447–4452.

Fenelon, K., B. Xu, C.S. Lai, J. Mukai, S. Markx, K.L. Stark, P.K. Hsu, W.B. Gan, G.D. Fischbach, A.B. MacDermott, M. Karayiorgou, and J.A. Gogos. 2013. The pattern of cortical dysfunction in a mouse model of a schizophrenia-related microdeletion. *J Neurosci*. 33:14825–14839.

Fischbach, G.D. 1970a. The different effect of neuromuscular inactivity and muscle atrophy on speed of contraction. *Exp Neurol*. 28:189–190.

Fischbach, G.D. 1970b. Synaptic potentials recorded in cell cultures of nerve and muscle. *Science*. 169:1331–1333.

Fischbach, G.D. 1972. Synapse formation between dissociated nerve and muscle cells in low density cell cultures. *Dev Biol*. 28:407–429.

Fischbach, G.D. 1987. An introduction to molecular approaches in studies of neuronal development and synapse formation. *Res Publ Assoc Res Nerv Ment Dis*. 65:143–153.

Fischbach, G.D. 1992. Mind and brain. *Sci Am*. 267:48–57.

Fischbach, G.D. 2006. Schizophrenia: signals from the other side. *Nat Med*. 12:734–735.

Fischbach, G.D. 2007. NRG1 and synaptic function in the CNS. *Neuron*. 54:495–497.

Fischbach, G.D., H. Aratake, G. Corfas, D.L. Falls, A. Goodearl, and K.M. Rosen. 1994. Trophic interactions at developing synapses. *Prog Clin Biol Res*. 390: 173–190.

Fischbach, G.D., D.K. Berg, S.A. Cohen, and E. Frank. 1976. Enrichment of nerve-muscle synapses in spinal cord–muscle cultures and identification of relative peaks of ACh sensitivity at sites of transmitter release. *Cold Spring Harb Symp Quant Biol*. 40:347–357.

Fischbach, G.D., and S.A. Cohen. 1973. The distribution of acetylcholine sensitivity over uninnervated and innervated muscle fibers grown in cell culture. *Dev Biol*. 31:147–162.

Fischbach, G.D., S.A. Cohen, and M.P. Henkart. 1974. Trophic functions of the neuron. I. Development of neural connections. Some observations of trophic interaction between neurons and muscle fibers in cell culture. *Ann N Y Acad Sci*. 228:35–46.

Fischbach, G.D., and M.A. Dichter. 1974. Electrophysiologic and morphologic properties of neurons in dissociated chick spinal cord cell cultures. *Dev Biol*. 37:100–116.

Fischbach, G.D., K. Dunlap, A. Mudge, and S. Leeman. 1981. Peptide and amine transmitter effect on embryonic chick sensory neurons in vitro. *Adv Biochem Psychopharmacol*. 28:175–188.

Fischbach, G.D., D. Fambrough, and P.G. Nelson. 1973. A discussion of neuron and muscle cell cultures. *Fed Proc*. 32:1636–1642.

Fischbach, G.D., and R.L. Fischbach. 2004. Stem cells: science, policy, and ethics. *J Clin Invest*. 114:1364–1370.

Fischbach, G.D., E. Frank, T.M. Jessell, L.L. Rubin, and S.M. Schuetze. 1978. Accumulation of acetylcholine receptors and acetylcholinesterase at newly formed nerve-muscle synapses. *Pharmacol Rev*. 30:411–428.

Fischbach, G.D., and Y. Lass. 1978a. Acetylcholine noise in cultured chick myoballs: a voltage clamp analysis. *J Physiol*. 280:515–526.

Fischbach, G.D., and Y. Lass. 1978b. A transition temperature for acetylcholine channel conductance in chick myoballs. *J Physiol*. 280:527–536.

Fischbach, G.D., and C. Lord. 2010. The Simons Simplex Collection: a resource for identification of autism genetic risk factors. *Neuron*. 68:192–195.

Fischbach, G.D., and G.M. McKhann. 2001. Cell therapy for Parkinson's disease. *N Engl J Med*. 344:763–765.

Fischbach, G.D., M. Nameroff, and P.G. Nelson. 1971. Electrical properties of chick skeletal muscle fibers developing in cell culture. *J Cell Physiol*. 78:289–299.

Fischbach, G.D., and N. Robbins. 1969. Changes in contractile properties of disused soleus muscles. *J Physiol*. 201:305–320.

Fischbach, G.D., and N. Robbins. 1971. Effect of chronic disuse of rat soleus neuromuscular junctions on postsynaptic membrane. *J Neurophysiol*. 34:562–569.

Fischbach, G.D., and K.M. Rosen. 1997. ARIA: a neuromuscular junction neuregulin. *Annu Rev Neurosci*. 20:429–458.

Fischbach, G.D., and S.M. Schuetze. 1980. A post-natal decrease in acetylcholine channel open time at rat end-plates. *J Physiol*. 303:125–137.

Fischbach, R.L., and G.D. Fischbach. 2005. The brain doesn't lie. *Am J Bioeth*. 5:54–55; discussion W55.

Fischbach, R.L., and G.D. Fischbach. 2008. Neuroethicists needed now more than ever. *Am J Bioeth*. 8:47–48.

Fischbach, R.L., M.J. Harris, M.S. Ballan, G.D. Fischbach, and B.G. Link. 2015. Is there concordance in attitudes and beliefs between parents and scientists about autism spectrum disorder? *Autism*. doi: 10.1177/1362361315585310.

Ford, B.D., B. Han, and G.D. Fischbach. 2003a. Differentiation-dependent regulation of skeletal myogenesis by neuregulin-1. *Biochem Biophys Res Commun*. 306:276–281.

Ford, B.D., Y. Liu, M.A. Mann, R. Krauss, K. Phillips, L. Gan, and G.D. Fischbach. 2003b. Neuregulin-1 suppresses muscarinic receptor expression and acetylcholine-activated muscarinic K+ channels in cardiac myocytes. *Biochem Biophys Res Commun*. 308:23–28.

Ford, B.D., J.A. Loeb, and G.D. Fischbach. 1999. Neuregulin stimulates DNA synthesis in embryonic chick heart cells. *Dev Biol*. 214:139–150.

Frank, E., and G.D. Fischbach. 1977. ACh receptors accumulate at newly formed nerve-muscle synapses in vitro. *Soc Gen Physiol Ser*. 32:285–291.

Frank, E., and G.D. Fischbach. 1979. Early events in neuromuscular junction formation in vitro: induction of acetylcholine receptor clusters in the postsynaptic membrane and morphology of newly formed synapses. *J Cell Biol*. 83:143–158.

Fukuda, J., G.D. Fischbach, and T.G. Smith, Jr. 1976a. A voltage clamp study of the sodium, calcium and chloride spikes of chick skeletal muscle cells grown in tissue culture. *Dev Biol*. 49:412–424.

Fukuda, J., M.P. Henkart, G.D. Fischbach, and T.G. Smith, Jr. 1976b. Physiological and structural properties of colchicine-treated chick skeletal muscle cells grown in tissue culture. *Dev Biol*. 49:395–411.

Goodearl, A.D., A.G. Yee, A.W. Sandrock, Jr., G. Corfas, and G.D. Fischbach. 1995. ARIA is concentrated in the synaptic basal lamina of the developing chick neuromuscular junction. *J Cell Biol*. 130:1423–1434.

Han, B., and G.D. Fischbach. 1999a. Processing of ARIA and release from isolated nerve terminals. *Philos Trans R Soc Lond B Biol Sci*. 354:411–416.

Han, B., and G.D. Fischbach. 1999b. The release of acetylcholine receptor inducing activity (ARIA) from its transmembrane precursor in transfected fibroblasts. *J Biol Chem*. 274:26407–26415.

Harris, D.A., D.L. Falls, R.M. Dill-Devor, and G.D. Fischbach. 1988. Acetylcholine receptor-inducing factor from chicken brain increases the level of mRNA encoding the receptor alpha subunit. *Proc Natl Acad Sci U S A*. 85:1983–1987.

Harris, D.A., D.L. Falls, and G.D. Fischbach. 1989. Differential activation of myotube nuclei following exposure to an acetylcholine receptor-inducing factor. *Nature*. 337:173–176.

Harris, D.A., D.L. Falls, F.A. Johnson, and G.D. Fischbach. 1991. A prion-like protein from chicken brain copurifies with an acetylcholine receptor-inducing activity. *Proc Natl Acad Sci U S A*. 88:7664–7668.

Hourani, B.T., B.F. Torain, M.P. Henkart, R.L. Carter, V.T. Marchesi, and G.D. Fischbach. 1974. Acetylcholine receptors of cultured muscle cells demonstrated with ferritin-alpha-bungarotoxin conjugates. *J Cell Sci*. 16:473–479.

Hume, R.I., L.W. Role, and G.D. Fischbach. 1983. Acetylcholine release from growth cones detected with patches of acetylcholine receptor-rich membranes. *Nature*. 305:632–634.

Jacobs, M.P., G.D. Fischbach, M.R. Davis, M.A. Dichter, R. Dingledine, D.H. Lowenstein, M.J. Morrell, J.L. Noebels, M.A. Rogawski, S.S. Spencer, and W.H. Theodore. 2001. Future directions for epilepsy research. *Neurology*. 57:1536–1542.

Jessell, T.M., R.E. Siegel, and G.D. Fischbach. 1979. Induction of acetylcholine receptors on cultured skeletal muscle by a factor extracted from brain and spinal cord. *Proc Natl Acad Sci U S A*. 76:5397–5401.

Lass, Y., and G.D. Fischbach. 1976. A discontinuous relationship between the acetylcholine-activated channel conductance and temperature. *Nature*. 263: 150–151.

Liu, Y., B. Ford, M.A. Mann, and G.D. Fischbach. 2001. Neuregulins increase alpha7 nicotinic acetylcholine receptors and enhance excitatory synaptic transmission in GABAergic interneurons of the hippocampus. *J Neurosci*. 21:5660–5669.

Liu, Y., B.D. Ford, M.A. Mann, and G.D. Fischbach. 2005. Neuregulin-1 increases the proliferation of neuronal progenitors from embryonic neural stem cells. *Dev Biol*. 283:437–445.

Loeb, J.A., and G.D. Fischbach. 1995. ARIA can be released from extracellular matrix through cleavage of a heparin-binding domain. *J Cell Biol*. 130:127–135.

Loeb, J.A., and G.D. Fischbach. 1997. Neurotrophic factors increase neuregulin expression in embryonic ventral spinal cord neurons. *J Neurosci*. 17:1416–1424.

Loeb, J.A., A. Hmadcha, G.D. Fischbach, S.J. Land, and V.L. Zakarian. 2002. Neuregulin expression at neuromuscular synapses is modulated by synaptic activity and neurotrophic factors. *J Neurosci*. 22:2206–2214.

Loeb, J.A., T.S. Khurana, J.T. Robbins, A.G. Yee, and G.D. Fischbach. 1999. Expression patterns of transmembrane and released forms of neuregulin during spinal cord and neuromuscular synapse development. *Development*. 126:781–791.

Loeb, J.A., E.T. Susanto, and G.D. Fischbach. 1998. The neuregulin precursor proARIA is processed to ARIA after expression on the cell surface by a protein kinase C-enhanced mechanism. *Mol Cell Neurosci*. 11:77–91.

Mann, M.A., S. Das, J. Zhang, M. Wagner, and G.D. Fischbach. 2006. Neuregulin effect on quantal content dissociated from effect on miniature endplate potential amplitude. *J Neurophysiol*. 96:671–676.

Mann, M.A., D.M. Knipe, G.D. Fischbach, and B.N. Fields. 2002. Type 3 reovirus neuroinvasion after intramuscular inoculation: direct invasion of nerve terminals and age-dependent pathogenesis. *Virology*. 303:222–231.

Martinou, J.C., D.L. Falls, G.D. Fischbach, and J.P. Merlie. 1991. Acetylcholine receptor-inducing activity stimulates expression of the epsilon-subunit gene of the muscle acetylcholine receptor. *Proc Natl Acad Sci USA*. 88:7669–7673.

Mudge, A.W., S.E. Leeman, and G.D. Fischbach. 1979. Enkephalin inhibits release of substance P from sensory neurons in culture and decreases action potential duration. *Proc Natl Acad Sci U S A*. 76:526–530.

O'Brien, R.J., and G.D. Fischbach. 1986a. Characterization of excitatory amino acid receptors expressed by embryonic chick motoneurons in vitro. *J Neurosci*. 6:3275–3283.

O'Brien, R.J., and G.D. Fischbach. 1986b. Excitatory synaptic transmission between interneurons and motoneurons in chick spinal cord cell cultures. *J Neurosci*. 6:3284–3289.

O'Brien, R.J., and G.D. Fischbach. 1986c. Isolation of embryonic chick motoneurons and their survival in vitro. *J Neurosci*. 6:3265–3274.

O'Brien, R.J., and G.D. Fischbach. 1986d. Modulation of embryonic chick motoneuron glutamate sensitivity by interneurons and agonists. *J Neurosci*. 6:3290–3296.

O'Brien, R.J., S. Kamboj, M.D. Ehlers, K.R. Rosen, G.D. Fischbach, and R.L. Huganir. 1998. Activity-dependent modulation of synaptic AMPA receptor accumulation. *Neuron*. 21:1067–1078.

Robbins, N., and G.D. Fischbach. 1971. Effect of chronic disuse of rat soleus neuromuscular junctions on presynaptic function. *J Neurophysiol*. 34:570–578.

Role, L.W., and G.D. Fischbach. 1987. Changes in the number of chick ciliary ganglion neuron processes with time in cell culture. *J Cell Biol*. 104:363–370.

Role, L.W., V.R. Matossian, R.J. O'Brien, and G.D. Fischbach. 1985. On the mechanism of acetylcholine receptor accumulation at newly formed synapses on chick myotubes. *J Neurosci*. 5:2197–2204.

Role, L.W., D.G. Roufa, and G.D. Fischbach. 1987. The distribution of acetylcholine receptor clusters and sites of transmitter release along chick ciliary ganglion neurite-myotube contacts in culture. *J Cell Biol*. 104:371–379.

Rosen, K.M., A.W. Sandrock, Jr., A.D. Goodearl, J.A. Loeb, and G.D. Fischbach. 1996. The role of neuregulin (ARIA) at the neuromuscular junction. *Cold Spring Harb Symp Quant Biol*. 61:427–434.

Rubin, L.L., S.M. Schuetze, and G.D. Fischbach. 1979. Accumulation of acetylcholinesterase at newly formed nerve–muscle synapases. *Dev Biol*. 69:46–58.

Rubin, L.L., S.M. Schuetze, C.L. Weill, and G.D. Fischbach. 1980. Regulation of acetylcholinesterase appearance at neuromuscular junctions in vitro. *Nature*. 283:264–267.

Sandrock, A.W., Jr., S.E. Dryer, K.M. Rosen, S.N. Gozani, R. Kramer, L.E. Theill, and G.D. Fischbach. 1997. Maintenance of acetylcholine receptor number by neuregulins at the neuromuscular junction in vivo. *Science*. 276:599–603.

Sandrock, A.W., Jr., A.D. Goodearl, Q.W. Yin, D. Chang, and G.D. Fischbach. 1995. ARIA is concentrated in nerve terminals at neuromuscular junctions and at other synapses. *J Neurosci*. 15:6124–6136.

Schuetze, S.M., E.F. Frank, and G.D. Fischbach. 1978. Channel open time and metabolic stability of synaptic and extrasynaptic acetylcholine receptors on cultured chick myotubes. *Proc Natl Acad Sci U S A*. 75:520–523.

Siegel, R.E., and G.D. Fischbach. 1984. Muscarinic receptors and responses in intact embryonic chick atrial and ventricular heart cells. *Dev Biol*. 101:346–356.

Smilowitz, H., and G.D. Fischbach. 1978. Acetylcholine receptors on chick mononucleated muscle precursor cells. *Dev Biol*. 66:539–549.

Trinidad, J.C., G.D. Fischbach, and J.B. Cohen. 2000. The Agrin/MuSK signaling pathway is spatially segregated from the neuregulin/ErbB receptor signaling pathway at the neuromuscular junction. *J Neurosci*. 20:8762–8770.

Trussell, L.O., and G.D. Fischbach. 1989. Glutamate receptor desensitization and its role in synaptic transmission. *Neuron*. 3:209–218.

Trussell, L.O., L.L. Thio, C.F. Zorumski, and G.D. Fischbach. 1988a. Rapid desensitization of glutamate receptors in vertebrate central neurons. *Proc Natl Acad Sci U S A*. 85:4562–4566.

Trussell, L.O., L.L. Thio, C.F. Zorumski, and G.D. Fischbach. 1988b. Rapid desensitization of glutamate receptors in vertebrate central neurons. *Proc Natl Acad Sci U S A*. 85:2834–2838.

Tsui, H.C., J.B. Cohen, and G.D. Fischbach. 1990. Variation in the ratio of acetylcholine receptors and the Mr 43,000 receptor-associated protein in embryonic chick myotubes and myoblasts. *Dev Biol*. 140:437–446.

Usdin, T.B., and G.D. Fischbach. 1986. Purification and characterization of a polypeptide from chick brain that promotes the accumulation of acetylcholine receptors in chick myotubes. *J Cell Biol*. 103:493–507.

Vartanian, T., G. Corfas, Y. Li, G.D. Fischbach, and K. Stefansson. 1994. A role for the acetylcholine receptor-inducing protein ARIA in oligodendrocyte development. *Proc Natl Acad Sci USA*. 91:11626–11630.

Yee, A.G., G.D. Fischbach, and M.J. Karnovsky. 1978. Clusters of intramembranous particles on cultured myotubes at sites that are highly sensitive to acetylcholine. *Proc Natl Acad Sci U S A*. 75:3004–3008.

Zorumski, C.F., J. Yang, and G.D. Fischbach. 1989. Calcium-dependent, slow desensitization distinguishes different types of glutamate receptors. *Cell Mol Neurobiol*. 9:95–104.