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Tsinghua University, Taiwan, Physics (1970)
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Appointments:

Postdoctoral Fellow, Purdue University (1974–1976)
Assistant, Associate, Full Professor, University of California, Irvine (1976–1985)
Professor of Research, Yale University School of Medicine (1985–1988)
Professor of Biological Sciences, Columbia University (1988–1995)
Stephen Kuffler Professor of Neurobiology, University of California, San Diego (1996–2000)
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Member, Academia Sinica. Taiwan (2000)
Fellow, American Association for Advancement of Science (2001)
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P.R. China International Science and Technology Cooperation Award (2005)
U.S. National Academy of Sciences, member (2009) and international member (2018)
Qiu-shi Distinguished Scientist Award, Hong Kong (2010)
Chinese Academy of Sciences, foreign member (2011) and regular member (2018)
Docteur Honoris Causa, Hong Kong University of Science and Technology (2014)
Founding Member, Hong Kong Academy of Science (2014)
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Mu-ming Poo began his research as a biophysicist studying protein mobility in cell membranes and receptor localization mechanisms during synaptogenesis. He later developed a method for assaying axon growth in response to guidance factors in vitro and showed that the attractive vs. repulsive growth cone turning induced by a guidance factor depends on the internal cyclic nucleotide concentrations. He also studied the role of neurotrophins and electrical activity in synaptic plasticity and characterized the time window for spike timing-dependent plasticity. His recent interest is to use nonhuman primates to study cognitive functions and brain disorders.

Mu-ming Poo

My Family

My family belongs to a subgroup of Han Chinese known as "Hakka," or guest families, who migrated in ancient times from the Yellow River basin to Southern China as a result of wars and famines. The ancestors of the Poo family settled in a village known as Sanheba (three-river village) in eastern Guangdong providence. The Mei-jiang River flows south from Sanheba to the South China Sea through the port city of Shantou, where many Chinese immigrants embarked on their journeys to Southeast Asia. My grandfather was a boatman on a barge used to transport goods along the Mei-jiang River. He lost his life during a typhoon season, leaving my grandmother and five children. Grandmother made a meager living by selling vegetables grown in the family farm, and with the help of relatives, managed to send the kids one-by-one to attend schools in Shantou. Remarkably, all five children eventually received a college education. My father was the third child, and he was helped throughout his school years by his big brother, a self-made man who worked his way to become a high-ranking official in the provincial government.

My father Liang-shao Poo attended an elite Zhongshan High School in Guangzhou and graduated from Jiaotong University in Shanghai in 1938, with a degree in aeronautical engineering. He joined the Chinese Air Force, along with many of his classmates, at the outbreak of the Sino-Japanese war. My uncle Liang-lou Poo graduated from the Chinese Air Force Academy and became a fighter plane pilot. During a social gathering, my uncle Poo introduced my father to a pilot friend's sister Shou-yu Li, who later became my mother. In 1943, my father was sent by the Chinese government to the United States for advanced studies in aeronautical engineering. He spent two years in the Brooklyn Polytechnic Institute in New York, obtained a master's degree, and received two more years of practical training in the U.S. aero-industry before returning to Nanjing in 1947 to take a job in an Air Force factory producing propeller engines. Under the order of the Nationalists government, he left with the factory to Taiwan before I was born on October 31, 1948, in Nanjing's Air Force Hospital, under candlelight during a power outage. The light came back soon after I was delivered. The doctor suggested that I should be named "light" (ming). I was thus named Mu-ming, with "mu" (meaning "admire") the family middle name. Two months later, as Mao's People's Liberation Army was approaching Nanjing, my mother carried me in her arms and, together with my sister and grandmother, boarded an overcrowded boat from Nanjing up the Yangtze River to Wuhan, where they managed to get train tickets to Guangzhou and then a ship to Taiwan.

The family settled in a small coast town Qingshui in central Taiwan, where my father was appointed as the head of an airplane engine factory. He was a diligent and austere manager at work and a strict father at home. I was greatly influenced by his strong self-discipline and work ethic. I recall that he was always working at the desk in the evenings, correcting proofs of Chinese translations of engineering manuals for his factory workers. He once taught me how to use a slide rule he had kept since his student days, but I never found a chance to use the skill. My mother had a college-level education but did not have her own job. She kept the family in good order. I excelled in a local primary school and was later accepted into the Taichung City's First Middle School for six years of middle school education. The school had an excellent library, with a large collection of ancient and modern Chinese books, from which I developed a strong interest in literature and history. To borrow books from the library, we searched for books in a catalog in the library hall and sent in a slip with the priority list to the staff for fetching the books from the storage room. I still have vivid memories of the face of a female staff, with her smiling and sometimes suspicious looks at me, a kid with a head barely reaching the check-out counter in the beginning, who grew into a tall young man in six years.

I had two siblings, an elder sister Mu-rong (Alice P. Lin) born in Chengdu during wartime, and a younger brother Mu-chou born in Taiwan. Mu-rong had a strong influence on my intellectual development; she had a literary bend and bought most of the books in the family. She later received a doctoral degree in social work from University of Michigan, had a distinguished career in public health, and once served as the deputy mental health commissioner for New York State during Mario Cuomo's administration. She died in 2014 of ovarian cancer. My brother Mu-chou received his doctorate in Near Eastern Studies from Johns Hopkins University. He excelled as an Asian scholar specializing in Egyptology and comparative ancient history. He is still teaching in the History Department of the Chinese University of Hong Kong. I was married in 1974 to Wen-jen Hwu, a chemist-turned-oncologist specializing in melanoma treatment who worked for many years at the MD Anderson Cancer Center. We divorced in 1994. Wen-jen and I have two daughters, both born in the United States. Ai-jen graduated from Columbia University with a major in women's studies, and she became an accomplished labor organizer. She is now the president of the National Domestic Workers Alliance and director of Caring Across Generations. Ting graduated from Barnard College majoring in film studies and has a successful career as an editor and director of documentary films, with an Oscar-winning record. I remarried in 2003 to Yang Dan, a former student at Columbia who became a distinguished neurobiologist in systems neuroscience and sleep research. Yang has been a close partner in my scientific journey over the past two decades.

Studying Physics at Tsinghua University

When the time came for my college education, I followed my father's advice and entered the Department of Nuclear Engineering at Taiwan's Tsinghua University in 1966. This was my top choice at the time, because nuclear energy appeared to be a thing of the future, and Taiwan was planning to build its first nuclear power plant. However, I realized during my freshman year that I should learn more basic physics for my college education, so I applied to be transferred to the Physics Department. Unsatisfied with the way physics was taught by young instructors who mostly read their lecture notes, my fellow student Wu (Alex) Chao and I decided to learn physics ourselves by studying Richard Feynman's Lectures on Physics. Alex was much better at understanding the material, and catching up to his pace was my daily goal. Alex later became a distinguished accelerator physicist at Stanford and once headed the theory division for the ill-fated superconducting supercollider. Overall, my best physics education at Tsinghua was studying the three volumes of the Feynman Lectures with Alex Chao.

One late winter night in 1968, while passing through the library hall, I noticed James Watson's *Double Helix* in the new book exhibition window. Having heard of Watson and Crick's Nobel Prize work on DNA structure, I was the first to check out that book. It was the first science book that struck my fancy. I learned how great science was done and how it could be such an exciting adventure. In the early summer of 1968, a physics professor Yi-yan Lee called me into his office. He had received an invitation from the Hsu Foundation in Hong Kong to help launch a publishing project in Taiwan—translation of popular science books from English into Chinese. The first book he selected was George Gamow's *Mr. Tompkins in Wonderland*, a book on discoveries in modern physics that Mr. Tompkins learned in his dreams. Lee said that since I had learned some physics and seemed to be good in English, I should translate the book into Chinese, with the condition that it had to done by the end of summer. I took the job with trepidation. It was quite difficult for a 19-year-old kid to miss all the summer vacation fun with friends and spend 10 hours a day at the desk for three months. I did finish the job by the end of the summer, with some help from my friends Alex Chao and Ning Hsieh. When the next summer came, Lee handed me two volumes of a book on *Interesting Experiments* to translate, a manual for middle-school kids to do physics and chemistry experiments at home, using readily available household materials. All three books were published by the Hsu Foundation during 1968–1969. Gamow's book was reprinted many times in Taiwan and was on the recommended summer reading list for high-school students for many years. When Gamow's book was ready for publication, I wrote to Professor Gamow, who lived in Colorado then, requesting a preface for the Chinese version of his famed book. Sadly, I received a reply from Mrs. Gamow saying that he had passed away earlier that year. My translation

work had earned me a handsome fee that allowed me to buy a good-quality used violin made in Czechoslovakia. The real benefit, as I realized later, was the cultivation of self-discipline. I had no problem later in life to work at the lab bench for long hours. By translating *Interesting Experiments*, I probably also developed a knack for doing experiments with simple means and making usable gadgets from junk materials in the lab, a skill that facilitated the pace of my doctoral research.

Graduate Studies at Johns Hopkins

After one year of compulsory military service as an instructor in the Taiwanese Army, I arrived in Baltimore in late August of 1971 for graduate studies in physics at Johns Hopkins University. I was greatly impressed by the beautiful Homewood campus and the high-quality physics faculty, but was immediately stressed by the duties of being a teaching assistant for Physics 101. With an English proficiency barely sufficient for conducting a conversation, I had to stand in front of a group of students to answer questions about lecture materials and course assignments. The first-year graduate courses in quantum mechanics, classical electrodynamics, and linear algebra were no less demanding. When the spring term arrived, I applied to take the doctoral qualifying examination. This was quite unusual at the time, because most students took the qualifying exam after a few years into their doctoral thesis work. Nevertheless, my request was granted. The exam consisted of a series of meetings with individual faculty members, answering questions in various areas in physics, and ended with a general oral exam in front of a group of professors. Many questions were quite unexpected. For example, would a large ball and a small ball fall to the ground at the same time if the Pisa Tower were filled with oil? How do you calculate the true position of a star, given light refraction by the atmosphere? What is the chance of hitting a tree if you fire a bullet into the forest? I soon found out that the examiners just want to know how I thought through the problems rather than the answers, because I was often stopped half-way into my calculations.

The Hopkins' Physics Department had a nice tradition of allowing graduate students to do their thesis work with any faculty member on campus. After passing the qualifying exam, I began to think about potential thesis projects. Having read Watson's story of DNA structure, I thought X-ray crystallography may be a good choice. On an early spring day in 1972, I ventured into the office of Warner Love, then chair of the Jenkins Department of Biophysics and a crystallographer, and asked for his advice. He pulled out a reprint from his drawer, a paper by Francis Crick on the solution of helical structure using X-ray diffraction data, and asked me to read it and come back to talk afterward. I read the paper loaded with Bessel functions and found it rather boring. Instead of going back to Professor Love, I began sitting in on a course on General Physiology given by a biophysicist Richard

Cone. I found the lectures fascinating—how complex physiological processes could be understood with simple physics and mathematics. After the course was over, I asked Richard whether I could work in his laboratory during the summer, and he kindly agreed. When the summer ended, my lab work was satisfactory enough for Richard to take me on as a graduate student.

Richard was a brilliant physicist-turned-biophysicist. He gained an early fame for dissecting the cellular origin of early receptor potentials in the electroretinogram. When I joined his laboratory in the fall of 1972, he was working on two problems. First, to find evidence for the "calcium hypothesis of phototransduction"—light activation of rhodopsin in the disk membrane of photoreceptors causes calcium release from the discs, which in turn induces a photoceptor potential by blocking sodium channels in the plasma membrane. Although I did not participate in the phototransduction work, I was exposed to the exciting atmosphere and intense competition among the labs of Richard Cone, Bill Hagins, and others. Several years later, the "internal transmitter" turned out to be cyclic guanosine monophosphate (cGMP), a rather unlikely candidate at the time. The second area Richard worked on—rhodopsin mobility within the disk membrane—brought him the greatest glory. It was a time when membrane biology was undergoing a mini-revolution as people realized the fluidity of cell membranes. In an elegant experiment, Richard showed that rhodopsin undergoes rapid rotational diffusion in the photoreceptor disc membrane, suggesting a local membrane viscosity of about 1 poise (Cone, 1972).

After joining Richard's lab, I was assigned the job of finding out whether rhodopsin could diffuse laterally in the disc membrane and, if so, whether the bulk membrane viscosity experienced by rhodopsin's lateral movement is the same as that reported by its local rotational motion. Richard suggested a conceptually very simple experiment: bleach isolated a rod photoreceptor outer segment on one side to create a gradient of unbleached rhodopsin molecules in the plane of disc membranes, which were all aligned perpendicularly to the rod axis. Then, by measuring the rate of redistribution of rhodopsin absorbances between the bleached and unbleached sides, I could determine rhodopsin's translational diffusion coefficient. It took me a few weeks to build a small motor-driven gadget that could be fitted into the cavity of a microspectrophotometer. The 1- to 2-μm slit for the bleaching and measuring light was formed by the edges of two razor blades and focused through an objective lens onto the photoreceptor outer segment. Alternating absorbance measurements on two sides of the outer segment was made simply by motor-driven movements of a tilted glass slide in and out of the light beam. Within a few months, I obtained convincing evidence for rapid lateral redistribution of bleached and unbleached rhodopsin molecules in the disc membrane.

To deduce the diffusion coefficient of rhodopsin molecules from the rate of their redistribution was not straightforward, because the disc membranes

have many deep fissures that impose complex boundary conditions for rhodopsin diffusion. My math friend told me that an analytical solution of a diffusion equation for such complex geometry would be a nightmare, and this was a time before one could perform Monte Carlo simulations of diffusion processes using a desktop computer. Eventually, I got around this problem by performing an "analog" computation—assessing the effect of similar fissures on the rate of heat diffusion in metal discs, after one side of the disc was dipped quickly into hot water. Because heat diffusion and molecular diffusion follow the same equation, I could directly measure the effect of disc geometries on the rate of heat diffusionas compared with the rate in a disc with analytically solvable geometry. I thus deduced the lateral diffusion coefficient of rhodopsin in the frog photoreceptor disc membrane to be 3.5 ± 1.5 cm^2/s. Based on the Stokes–Einstein equation, the bulk viscosity of the cell membrane experienced by rhodopsin was about 1 poise (Poo and Cone, 1974), the same as that found by Richard for its rotational diffusion. This diffusion coefficient for rhodopsin later became the benchmark value for "free" lateral diffusion of membrane proteins, because there is no apparent cytoplasmic constraint on rhodopsin's motion. In the absence of a bleachable chromophore like that in rhodopsin, the photobleaching method was later used for measuring diffusion of membrane proteins with fluorescent ligands attached. Fluorescence recovery after photobleaching (FRAP) is now a standard method used to study protein diffusion in cells.

I gave my doctoral thesis defense in December 1973 to a committee assembled by the Physics Department, with some biologists as members. This was about two and half years since I began my graduate study at Hopkins. A clear downside of such a short duration was the lack of extensive graduate training in either physics or biology, and I had no idea about what to do after receiving my degree. Nevertheless, despite my short stay in his lab, Richard Cone had a profound influence on the taste and style of my later research—to ask important questions and use the simplest experimental approach to answer them. Quick and simple approaches often yield insight upon which more sophisticated experiments and analysis can be developed later.

Searching for Exciting Biology at the Marine Biological Laboratory

When the summer of 1974 came, Richard suggested that I should go to the Marine Biological Laboratory (MBL) in Woods Hole to get a better feeling about what's exciting in biology before deciding what to do for my postdoc. Alan Fain kindly accommodated me in his newly established lab in MBL, and Ann Stuart from Harvard also offered me an opportunity to learn electrophysiology in her summer MBL lab. For three intense months at MBL, I spent most mornings sitting in the lectures of summer courses in neurobiology and physiology. I attended a memorable, and probably last, lecture by Kenneth

Cole on his invention of voltage clamp and John Dowling's lively physiology lectures, in which he drew the entire gut system on the board in seconds with chalk in both hands. Most afternoons I worked in Ann's laboratory, learning how to pull glass microelectrodes, make intracellular recordings from the axon of barnacle ventral eye photoreceptors, and measure their membrane properties that might account for the spread of photoreceptor potentials along the long axon. One late evening, while I was concentrating on the complicated barnacle dissection under dim red light, an old man came to the lab. He looked over my shoulder and asked a few questions, to which I did not reply with much attention. Sensing my unresponsiveness, he soon left. The next morning, I asked Ann about this old man, and Ann said "Oh, that must be Steve." This was the only time in my life that I could have talked with the great Stephen Kuffler, and I missed it!

Lab work was only a small part of life at MBL. In the evenings, there were always seminars and lectures in all fields, accompanied by lively exchanges. Thursday night seminars were mostly neurobiology related, often on unpublished results fresh out of the laboratory. Friday Evening Lectures were my favorite, because they were grand summaries of the scientists' lifelong work presented in a way understandable by an audience from diverse backgrounds. All these wonderful talks left me with the impression that biology is full of fascinating topics for young budding scientists to work on, but I still did not get a clear sense about what I should pursue. One afternoon near the end of the summer, I wandered through a new book exhibition in Lilly Hall and spotted a book *On Development* by J. T. Bonner, just published by Harvard University Press. I bought the book and read it in a few days. Bonner described the problem of development in two perspectives: an evolutionary view of development (later known as "evo-devo") and a molecular view on how newly synthesized materials could be localized in the developing system. I was greatly attracted to a section on localization mechanisms, in which he cited extensively the work of Lionel Jaffe, who showed that physical forces—light, gravity, pH gradient, and electric field—could polarize rhizoid outgrowth from a fertilized fucus egg, leading to polarized root/stem development of this brown alga. I was particularly fascinated by many of Jaffe's simple and elegant experiments. For example, to demonstrate the appearance of endogenous electric fields associated with polarized development, he piled a string of fertilized fucus eggs in a thin capillary tube, applied light from one end to polarize their development, and detected an emerging potential difference between two capillary ends with a voltmeter. Studying the role of physical forces in development sounded like an interesting area to work on. I soon applied to Lionel Jaffe for a postdoc position at Purdue University and was quickly accepted. Two decades later, when I reflected on how accidental reading of his book had started my research in developmental neurobiology, I wrote a letter to Professor Bonner, then retired from Princeton, thanking him for writing the book. He kindly replied with his characteristic modesty.

Postdoctoral Research at Purdue University

Lionel Jaffe was the most focused and dedicated researcher I have ever met. Trained at Caltech in the early 1950s, he spent his entire scientific career trying to figure out the mechanism that polarizes a fertilized fucus egg. Most interestingly, he found that an endogenous transcellular current appears during the polarization process—positive ions flow into the budding rhizoid and out of the other end of the egg. He thought that this current was responsible for driving the polarized movements of cytoplasmic substances. A relatively steady extracellular field around the polarizing egg due to the transcellular current was detectable with a vibrating platinum electrode. After arriving at West Lafayette, I began to use the "vibrating probe" to search for the existence of endogenous currents associated with neurite outgrowth in cultured neurons. The neurite's growth cone, however, is one hundred times smaller than a fucus egg, and the electrode became too noisy when its size was greatly reduced. After many months of technical work without avail, Lionel suggested that I reinvestigate whether applied electric fields could polarize neurite outgrowth—along the line of research on "galvanotropism" in the 1920s. The early findings of nerve galvanotropism were later discredited by Paul Weiss, who argued that the effect was indirectly induced by mechanical guidance of the plasma clots in the culture substrate that became oriented by the applied field. My new task was to see whether the electric field could orient nerve growth in a liquid culture medium. I soon found that extracellular electric field could indeed accelerate neurite growth and bend them toward the cathodal pole of the field.

Given my interest on membrane protein mobility, I thought that an electric field could polarize the distribution of growth-relevant membrane proteins by "electrophoresis." However, growth cones of the chick neurons I was using were too small for mapping protein distribution. I then noticed a paper published by Nick Spitzer on cultured *Xenopus* spinal neurons and myocytes (Spitzer and Lamborghini, 1976), which are much bigger than chick neurons. I wrote to Nick for help, and he graciously invited me to visit his lab in La Jolla to learn the culturing method. It was late spring of 1976. Young faculty members Nick Spitzer and Darwin Berg, fresh out of Steve Kuffler's neurobiology department, were setting up a vibrant neurobiology community on the Pacific Coast. I spent three days in Nick's lab, watching him making cultures on a simple lab bench, recording from single neurons in culture and in *Xenopus* larvae, and shouting with excitement whenever he got a cell. The lab atmosphere was drastically different from what I had experienced in the east. There was more fun and less inhibition. This impression must have influenced my decision to move to La Jolla two decades later.

After returning to West Lafayette, I began to study the effect of electric fields on membrane protein distribution in large cultured myocytes, together

with Ken Robinson, another postdoctoral fellow in the Jaffe lab. We decided to focus on a heterogenous group of membrane glycoproteins that bind to the lectin concanavalin A (Con A), which could be covalently labeled with a fluorescent ligand. We soon obtained convincing evidence of electric field-induced redistribution of Con A receptors in the myocyte membrane. We found marked accumulation of Con A receptors on the side of the myocyte facing the cathode within 30 min after applying a field of about 10 mV across a 10-μ-wide cell. We reported the results in an experimental paper (Poo and Robinson, 1977), with an accompanying paper on the theory of electrophoresis in membranes by Jaffe (1977). In the meantime, I also found that a steady electric field could strongly bias the direction of growth cone extension of *Xenopus* spinal neurons toward the cathode. The latter phenomenon was thoroughly examined by my graduate student Nilesh Patel a few years later (Patel and Poo, 1984).

University of California at Irvine

After two years of postdoctoral work in the Jaffe lab, I was expected to leave and find a faculty position. I applied for positions in a few universities and eventually received an offer of assistant professorship from the University of California–Irvine (UC Irvine) Physiology Department. Arriving in Irvine in the fall of 1976, I was given a small lab of about 200 square feet and $20,000 of start-up funds, which allowed me to buy a used Zeiss microscope, a Getting preamplifier, and a pair of micromanipulators.

Synaptic Clustering of ACh Receptors

With a humble beginning and diligent work by my first graduate student Normal Orida, we showed that steady extracellular electric fields could induce electrophoretic accumulation of surface nicotinic acetylcholine receptors (AChRs) on cultured myocytes and that accumulated receptors formed stable clusters without post-field back diffusion (Orida and Poo, 1978), suggesting an intrinsic propensity of AChRs for self-aggregation. We also made quantitative studies on electrophoretic and diffusion processes of other membrane proteins and lipids (Poo, 1981). By this time, I began to realize that a more biologically relevant problem was not how mobile membrane components are, but rather how they become localized in the membrane to perform their biological functions. Synaptic physiologists had long been interested in understanding how AChRs become clustered at postsynaptic muscle membrane during the formation of neuromuscular junctions. Two possibilities were then proposed: newly synthesized receptors in the cytoplasm are locally inserted into the postsynaptic membrane, or alternatively, initially randomly inserted receptors redistribute themselves through lateral diffusion and become trapped at the synaptic site by binding

to some aggregation-inducing locally secreted factors (named "agrin" or ARIA). Monroe Cohen and Christian Anderson at McGill University found that AChRs tagged with fluorescent α-bungarotoxin are initially dispersed in cultured *Xenopus* myocyte membrane, but became clustered to the site of neurite contact within an hour after the contact. Measurements with FRAP on α-bungarotoxin-labeled AChRs yielded a diffusion rate too slow to account for the neurite-induced AChR clustering, possibly because of the impediment by the bulky fluorescent tag on AChRs. How could one measure the diffusion of native AChRs without a fluorescent tag? This problem puzzled me for some time. During one sleepless night lying in bed, a simple idea jumped to mind—just locally inactivate some surface AChRs with α-bungarotoxin and monitor the recovery of local ACh sensitivity by iontopheretic application of ACh pulses, as native functional AChRs diffuse into the inactivated area. I did the experiment next morning, and it worked beautifully. Within a few weeks, enough data were collected to show that the diffusion rate of native AChRs is much faster than that based on FRAP measurements, and it is sufficient to account for AChR clustering through lateral redistribution (Poo, 1982). In later years I always hoped that sleepless nights would bring some useful thoughts, but that rarely happened!

Transmitters Secretion from Growth Cones

In the early 1980s, the appearance of gigaohm-seal patch clamp method allowed measurements of single-ion channel currents in membrane patches excised from cells. At the time, a talented physiologist, Steve Young, joined my lab for postdoctoral research, and he built a patch clamp amplifier by following the circuit diagram and specifications described in the paper published in *Pflugers Archiv* by Hamill et al. (1981). Using this patch clamp, Steve did two interesting experiments. First, by on-cell patch recording from cultured myocytes, he showed that AChRs in the electrophoresis-induced clusters in the myocyte membrane had a longer mean channel opening time than those dispersed in the same membrane (Young and Poo, 1983a), indicating that ion channel properties depend on their state of clustering and probably the local lipid environment. The second experiment was more novel. Using an outside-out patch of myocyte membrane as a "sniffer" for ACh, which activates single ACh channels in the patch, Steve found that the growth cone of *Xenopus* spinal neurons spontaneously secretes pulses of ACh. A few weeks later, we heard from people returning from the Society for Neuroscience Annual Meeting that Gerald Fischbach announced in a lecture during the meeting that his lab had discovered ACh secretion from the growth cone of cultured sympathetic neurons, using the same approach. I immediately called Gerald about our finding, and we agreed to coordinate the publication of the results, which appeared in two back-to-back papers in *Nature* a few months later (Hume et al., 1983; Young and Poo, 1983b). The significance

of growth cone secretion of transmitter before synaptogenesis, however, remains unclear. It could be a signal broadcast by the growth cone to its environment for some purpose, or just a by-product of exocytotic insertion of new membrane materials during nerve growth. Nevertheless, it indicates that the growth cone is ready to serve presynaptic functions, because firing the neuron could evoke Ca^{2+}-dependent ACh secretion from the growth cone (Sun and Poo, 1987).

Visits to Pasadena

Some of most memorable events during my Irvine period were associated with my trips to Pasadena, mostly to visit Chun-fang Wu, then a postdoc fellow in Seymore Benzer's lab at Caltech, whom I befriended during my Purdue days. Chun-fang and I met often during weekends in either Irvine or Pasadena to do fun experiments together. In one weekend, we devised a trick to dissociate and culture embryonic ganglionic neurons from third-instar *Drosophila* larvae with minimal damage to these small fragile cells (Wu et al., 1983), a method that became useful later for single-channel recording from *Drosophila* neurons. When I first met Seymore Benzer in his Caltech lab, our *in situ* electrophoresis papers had just appeared. The first thing Benzer said when he saw me was to point to these two articles and ask, "Is this real? Did you check Jaffe's math?" Seymore did not take for granted any published results, and he himself published very little. I learned from Chun-fang later that every paper from Benzer's lab was meticulously written and scrutinized before submission—people in the lab had to sit around a table for hours to go through every sentence of the paper, including those who were not coauthors. My last encounter with Seymore was in the 2004 Society for Neuroscience dinner reception honoring him as the first Gruber Prize recipient. During my brief talk as a member of the Prize Committee, he was visibly pleased by my comment that his paper-writing ritual was in active practice in my Institute of Neuroscience (ION) in Shanghai. Unfortunately, this writing ritual did not persist at ION. Fast publication without much concern about the quality of writing has become an overwhelming trend.

In his later years at Caltech, Max Delbruck became interested in membrane protein mobility. Together with a mathematician Philip Saffman, he published a modeling paper in which the membrane protein is depicted as a cylinder spanning the lipid bilayer and diffuses in an isotropic medium of a defined viscosity. This model nicely predicted the relationships between the rates of rotational and translational diffusion of rhodopsin that Richard and I had measured. The Saffman-Delbruck (1975) model later became the basis of many theoretical studies of protein mobility in cell membranes and lipid bilayers. When our work on *in situ* electrophoresis of membrane proteins appeared in 1977, he invited me to give a talk in his biophysics seminar

series. His enthusiasm in talking about science remained high, despite his deteriorating health. I talked with him in his office before the seminar and was quite moved when he started making calls to remind people about the seminar. The talk did not go smoothly because of Max's frequent interruptions. Nevertheless, I was congratulated afterward by Lily Jan, then a postdoc fellow in the Benzer lab, for being able to finish the talk. Max's interest in Brownian motion in cell membrane was probably related to his thinking on the reduction of dimensionality in biological diffusion processes. In an article published several years earlier (Adam and Delbruck, 1968), he put forth the idea that for a soluble substrate to reach its membrane-bound enzyme, it could first bind nonspecifically to the membrane surface and then diffuse on the surface until it found its target. He proposed that three-dimensional (3D) diffusion in solution coupled with two-dimensional (2D) diffusion on the membrane could make the target searching more efficient than the direct search by 3D diffusion alone. Back in Irvine, Mike McClosky and I were intrigued by this idea, began to gather the relevant data, and tried to determine whether the two-step process could be advantageous in various membrane-associated reactions. Unfortunately, we found that available data on lateral diffusion rates in membranes, membrane-substrate affinities, and the endogenous concentrations of enzymes and their aqueous substrates did not support the hypothesis (McCloskey and Poo, 1986).

Teaching in Postcultural Revolution China

I flew to Beijing on a cold December night in 1981, 33 years after I was carried by my mother to Taiwan. Through deserted and dimly lit streets, I was taken to the guesthouse of Beijing Medical College. Over the following month, I gave an intensive course for medical school instructors on cellular neurophysiology and membrane biology. This was part of an exchange program between Beijing Medical College and UC Irvine. The Chinese Ministry of Health had selected 40 basic science instructors from various medical schools for a month-long course. Most students in the course were older than me and had apparently suffered from a lack of academic activity due to the Cultural Revolution. Each day, I gave two to three hours of lectures in the morning and a research seminar in the afternoon on my own research or topics close to my interest. The students were eager to learn and very attentive in taking notes, but asked very few questions. My teaching activity in China continued in the following two summers, including a three-week membrane biophysics course in 1983 at Tsinghua University in Beijing for more than 200 students from many universities, and a two-week biophysics workshop in 1984 for 20 students at Nankai University. The Nanki workshop included laboratory sessions, in which the participants assembled a patch clamp amplifier and made the first single-channel recording in China, using cultured *Xenopus* myocytes.

The normalization of U.S.-China diplomatic relationship in 1979 was followed by active academic exchanges. Among the first group of scholars visiting the United States was a physicist, Nan-ming Zhao, from Tsinghua University, who spent a year at my Irvine lab in 1979 to learn membrane biophysics. Nan-ming often chatted with me about his self-appointed mission of restoring the Biology Department in Tsinghua. In the early 1950s, there was a massive reorganization of Chinese universities following the Soviet model. All of Tsinghua's science and humanity departments were moved to Peking University, making Tsinghua a pure engineering school. In June 1983, Tsinghua decided to restore the School of Science and approved Nan-ming's plan of establishing a new biology department, together with his proposal that I serve as the chair. I agreed to serve on a part-time basis, leaving daily administrative work to Nan-ming. With the first class entering in the fall of 1985, the immediate task was to establish the curriculum, recruit teaching faculty, and set up teaching laboratories. However, it was nearly impossible to begin any research program, because the funding could barely cover the staff salary and teaching expenses. Feeling powerless to make useful contributions, I resigned from the position after two years, but have kept my Tsinghua connection since then. A brilliant student in the first class of 1985, Yi-gong Shi later became a distinguished structural biologist at Princeton. He returned to Tsinghua in 2005 to lead Tsinghua's School of Life Science, which is now ranked among the top few in China.

Yale Medical School

While the research in my Irvine lab was making good progress, I had the unsettling feeling that I had not landed on an important problem. Sometime in 1984, Chuck Stevens came to Irvine to review the campus programs and mentioned his plan of setting up a Molecular Neurobiology Section at Yale, focusing on structure-function studies of ion channels that incorporate the latest molecular cloning and single-channel recording technologies. I thought more about this exciting area and called Chuck a few days later to ask about the possibility of joining his section. Chuck was enthusiastic about the idea but mentioned that he only had a nontenured research professorship to offer. Because I was already a tenured professor at Irvine, going to Yale would mean giving up a safe and comfortable job. Against the advice of some of my friends, I took the risky route to the east in the spring of 1985. The Molecular Neurobiology Section of Yale Medical School occupied a wing of the Tompkins Memorial Pavilion of Yale Hospital. The space given to the section was extremely small, with each laboratory occupying no more than a few hundred square feet. Chuck joked about the benefit of working in tight space—people are forced to get along with each other and to interact frequently. Indeed, during my three years at Yale, I had collaborated with Susan Amara, Michael Lerner, Steven Smith, and JoAnn Buchanan. Although not all work reached fruition, these collaborations greatly expanded my research interests.

Early Events of Synaptogenesis

Our finding of spontaneous ACh secretion from growth cones suggests that they are quite ready to serve presynaptic functions, and synaptogenesis could be a rapid event. After arriving in New Haven, we made intracellular recordings from cultured myocyte and waited for their contact of spinal neurons by growth cones to determine the time course of synaptogenesis. Later, we moved the loosely attached myocyte ("myoball") with the whole-cell recording pipette (the "chopstick-meatball" experiment) into contact with a growth cone. With the precise control of the timing of nerve-muscle contact, we found the immediate appearance of both spontaneous and evoked synaptic currents, with synaptic efficacy increasing gradually over the first few minutes after contact (Xie and Poo, 1986). JoAnn Buchanan from Stephen Smith's lab worked side by side with Yian Sun to prepare electron microscopic samples for the same nerve-muscle synapse that Yian had just recorded. They found that the early contacts capable of functional transmission were morphologically undifferentiated. It took a few hours before the emergence of anatomically recognizable synaptic structure, for example, active zone-like presynaptic specializations with vesicle clustering and postsynaptic membrane thickening associated with clusters of AChRs (Buchanan et al., 1989). Thus, anatomically undifferentiated contacts could be functionally active, and functional network activity in the developing brain could be more complex than that implied by morphologically identifiable synaptic connections.

Cell-Cell Recognition and Specificity of Synaptogenesis

Ever since Roger Sperry proposed the chemoaffinity hypothesis, synaptogenesis has been regarded as a specific process requiring matching of cell surface "recognition" molecules between pre- and postsynaptic neurons. Our results on neuromuscular synaptogenesis, however, suggest that functional synapses could be established between two cells if one could secrete transmitters and the other has transmitter receptors on the surface to respond. In fact, the hypothesized specific cell-cell recognition molecules for synaptogenesis have long been elusive, although many heterogenous surface adhesion proteins could impose some selectivity in the formation of stable contact between cells. Because transmitter receptors, transporters, and ion channels are a relatively cell type-specific group of membrane proteins for each neuron, all of them have large ectodomains for interactions with membrane proteins in the opposing cell surface. Thus, I proposed that these proteins could serve for selective adhesion and contact stabilization by interacting across the contact, and they themselves could also become trapped at the contact site for their intended functions (Poo, 1985). We made several attempts to test this hypothesis over the years, such as generating transmitter receptor ectodomain peptides to blocking selective neuronal adhesion, but we failed to obtain any convincing results.

Columbia University

My time at Yale ended abruptly in 1988, when Chuck Stevens decided to move to the Salk Institute, and the Section of Molecular Neurobiology was to be dissolved. I soon accepted a professorship offer from Columbia's Biology Department, where Darcey Kelley, Marty Chalfie, Eduardo Macagno, and Cyrus Levinthal had established an exciting neurobiology group. Added to the attraction of Columbia was the exciting atmosphere of the Morningside campus, New York Upper Westside, and a high-rise Riverside apartment overlooking the Hudson River.

Vesicular Transmitter Secretion from Non-neuronal Cells

The advent of molecular cloning had led to the identification of many proteins associated with synaptic vesicles and presynaptic membranes. One approach to understand how these proteins work together to achieve rapid calcium-dependent vesicular secretion is to reconstitute the synaptic vesicle secretion machinery in a non-neuronal cell through step-by-step introduction of neuron-specific presynaptic proteins and assay the functional contribution of each protein. Lacking any molecular expertise, I asked Paul Greengard at Rockefeller University to collaborate on reconstituting vesicular secretion in *Xenopus* oocytes, which was the standard expression system for testing the function of cloned ion channels. One of my first graduate students at Columbia, Janet Alder, spearheaded the collaboration. Janet's first experiment was to purify synaptic vesicles from the torpedo electric organ, inject them into *Xenopus* oocytes, and examine whether calcium-dependent secretion of acetylcholine could be detected. By expressing nicotinic AChRs in the plasma membrane of these oocytes, we could detect vesicular secretion by the appearance of events resembling miniature synaptic currents (minis). Indeed, Janet found that oocytes could show secretion of torpedo synaptic vesicles and the secretion depended on the expression of vesicle protein synaptophysin (Alder et al., 1992). Unexpectedly, another graduate student, Yang Dan, came to my office one day and showed me recordings of minis from isolated myocytes, when she loaded the whole-cell recording pipette with 1 mM ACh. Because the events were rather rare, my immediate reaction was that there must be some residual nerve terminals hidden underneath the myocyte. Yang soon came back with more recordings showing lots of minis, when ACh concentration in the pipette was elevated to 10 mM, and ACh secretion could be evoked by depolarizing the myocyte (Dan and Poo, 1992b). It appeared that cytoplasmic ACh had somehow hijacked the vesicle exocytosis pathway in the myocyte and that calcium-dependent vesicular exocytosis actually occurs in non-neuronal cells. Reconstitution of synaptic vesicle secretion could thus be accomplished in any cell type by expressing a few neuron-specific synaptic vesicle proteins that elevate the efficacy of

excitation-secretion coupling. Indeed, fibroblasts loaded with ACh exhibited more efficiently evoked ACh secretion by expressing a neuron-specific isoform of synaptotagmin (Morimoto et al., 1995).

Activity-Dependent Synaptic Competition

Our studies of synaptogenesis in nerve-muscle cultures led us to the activity-dependent synaptic competition during synapse development, a problem thoroughly reviewed by Jeff Lichtman and Dale Purves in their textbook *Principles of Neural Development* published in 1985. They also proposed a trophic factor hypothesis—synapses compete for trophic factors secreted at the synapse in an activity-dependent manner, with active synapses taking up more factors and surviving, whereas inactive synapses are deprived of the factors and eliminated. We began the study of synaptic competition by examining cultured *Xenopus* myocytes that were coinnervated by axons from multiple neurons. A graduate student, Yi-juan Lo, found that repetitively stimulation of one of the coinnervating neurons resulted in the suppression of synapses made by unstimulated ones, without affecting the efficacy of the stimulated synapse (Lo and Poo, 1991). This activity-induced hetero-synaptic depression of "inactive" synapses could represent a form of synaptic competition. Yang Dan later showed that such competition was mediated by cellular processes in the postsynaptic myocyte, because iontophoretic application of ACh pulses could substitute for the active nerve terminal to suppress inactive synapses. Coactivating the presynaptic neuron could also protect the synapse from the suppression by ACh pulses, but only when the neuronal activation occurred within 10 msec of ACh pulses (Dan and Poo, 1992a). This was a clear indication of the importance of spike timing in synaptic plasticity, a theme we later studied systematically at central glutamatergic synapses.

Neurotrophins as Synaptic Modulators

For many years, the distinguished neurophysiologist Ladislav Tauc organized a yearly meeting known as "Gif Lectures in Neurobiology" at Gif-sur-Yvette in suburban Paris, a wonderful place to meet neuroscientists from diverse fields. At a dinner party during the 1991 meeting, I met for the first time Nancy Ip, then a group leader in the newly founded company Regeneron in New York. Nancy was working on the cloning and physiological studies of various neurotrophic factors and their receptors. At the end of the dinner, Nancy and I decided to collaborate on studying the potential synaptic function of neurotrophins. A graduate student, Ann Lohof, performed the experiment and found that brain-derived neurotrophic factor (BDNF) and neurotrophin-3 indeed could markedly potentiate spontaneous and evoked synaptic currents at cultured *Xenopus* nerve-muscle synapses (Lohof et al., 1993). Other laboratories soon demonstrated the rapid potentiation effect of BDNF at glutamatergic synapses

in cell cultures and brain slices and the requirement of endogenously released BDNF for LTP induction at hippocampal CA1 synapses. In the subsequent decade, neurotrophins and their receptors, BDNF and TrkB in particular, were shown to be involved in a variety of brain disorders. Although originally identified as factors promoting neuronal survival and growth, neurotrophins are now regarded as neuromodulators for regulating neural circuit development and plasticity (Poo, 2001; Park and Poo, 2012).

Joining the "Synapse Club"

Our findings in nerve-muscle cultures had often prompted the question of their *in vivo* relevance and whether the neuromuscular synapse is a good model for central synapses. The turning point was perhaps a symposium on synaptic transmission held in Sweden in 1992. I gave a talk in front of a large group of intimidating synaptic physiologists, with Paul Greengard as the session chair. At some point during my talk, I proposed that the mature neuromuscular junction represents the ultimate synapse; it has attained the most reliable and potentiated state for synaptic transmission without the need for long-term potentiation (LTP), whereas all central synapses are immature synapses trying to climb up the ladder. Thus, the neuromuscular synapse should be given an honorary membership in the exclusive synapse club. The audience responded enthusiastically, and I talked beyond the allotted time. Paul later claimed that he had to pull me away from the platform, but that was an exaggeration. Per Andersen, the grandfather of LTP, was sitting in the second row. He stood up and stretched his arm to shake my hand as I stepped down from the podium, presumably a gesture of accepting the neuromuscular junction into the synapse club.

University of California at San Diego

My introduction to neurobiology was the textbook *From Neuron to Brain* (1976) by Stephen Kuffler and John Nichols. Kuffler was a scientist of great vision and broad interests, and the Harvard Neurobiology Department he created was the cradle for many leading neuroscientists of our generation. His character and impact were vividly portraited in a memorial volume "Steve," written collectively by his colleagues and students. In memory of Steve, an anonymous donor, presumably one of his Harvard progenies, established the Stephen Kuffler Chair of Neurobiology at the University of California–San Diego (UCSD). I was greatly honored to receive an offer from Nick Spitzer and colleagues to be the inaugural Kuffler Chair. I arrived in La Jolla at the end of 1995, along with a large group of graduate students and postdoctoral fellows.

Growth Cone Guidance

An initial focus of my UCSD lab was to study the chemotaxis hypothesis of growth cone guidance, proposed by Ramón y Cajal nearly a century ago.

Using the growth cone turning assay developed at Columbia (Lohof et al., 1992), two of my students, Hong-jun Song and Guo-li Ming, demonstrated that a gradient of BDNF was effective in causing chemotropic turning of *Xenopus* spinal neuron growth cones. By further studying second messengers that could be involved in the turning response, they made a remarkable discovery—the same BDNF gradient caused repulsive rather than attractive growth cone turning, when a competitive membrane-permeable analogue of cAMP or a protein kinase A inhibitor was present in the culture medium (Song et al., 1997). Thus, the growth cone's turning response depends on the level of cytoplasmic cAMP activity, and the attractive vs. repulsive effect of BDNF is not due to the intrinsic property of BDNF but rather is determined by the internal state of the neuron.

This was the time when the netrin and semaphorin (collapsin) families of guidance factors were identified as attractants and repellents for various types of neurons, respectively. Over a period of a few years, in collaboration with Marc Tessier-Lavigne, Christine Holt, Corey Goodman, and Lisa McKerracher, we showed that nearly all putative guidance factors could cause either attractive or repulsive turning of the growth cone, depending on the overall cytoplasmic level of either cAMP or cGMP (Song et al., 1998; Song and Poo, 2001). The magnitude of cytoplasmic Ca^{2+} gradients was also found to be critical in setting the direction of growth cone turning (Hong et al., 2000; Henley et al., 2004). These cytoplasmic second messengers are known to be downstream mediators and integrators for the effects of many extrinsic factors (e.g., cytokines, transmitters, neuromodulators, growth factors, adhesion molecules) and intrinsic factors (e.g., electrical activity) converging on the neuron. The growth cone thus integrates multiple signals received by a neuron, before its decision on attractive vs. repulsive turning in the gradient of a particular guidance factor.

There were nearly two decades of intense activity in the axon guidance field, stimulated largely by the discoveries of guidance factor families in the 1990s and findings of abnormal development of nerve connections resulting from genetic deletion or mutation of these factors. Although *in vitro* experiments had shown the attractive and repulsive actions of these factors when they were applied in a diffusive gradient, whether or not axon pathfinding *in vivo* depends on long-range gradients of secreted guidance factors remained unclear. Gradients of BDNF or netrin-1 bound to the culture substrate were shown to be equally effective as the diffusive gradient in causing growth cone turning (Mai et al., 2009), but secreted factors are unlikely to diffuse far from the secreted site because of their binding to the extracellular matrix. It is thus unclear how the spatiotemporal distribution of guidance factors within the tissue could be tightly regulated for guiding axons through the torturous terrain of the developing brain. Advances in spatial and temporal transcriptomic analysis may facilitate future studies of axon guidance *in vivo*, within the context of tissue morphogenesis.

Spike Timing-Dependent Plasticity

After moving to La Jolla, our study of synaptic competition was extended from cultured nerve-muscle synapses to retinotectal synapses *in vivo*. This transition was inspired by the work of Bill Harris and Christine Holt on the developing retinotectal system. With their help, two of my graduate students, Li Zhang and Huizhong Tao, were able to perform *in vivo* whole-cell recording from tectal neurons in unanesthetized *Xenopus* larvae and examine how retinal ganglion cell (RGC) axons coinnervating the same tectal neuron compete with one another during activity-dependent refinement of retinotectal connections. We initially had in mind the popular version of Hebb's hypothesis that correlated firing of neurons strengthens their synaptic connections. By varying the time intervals between the spiking of RGCs and tectal cells, we hoped to quantitatively assess the extent of correlation in pre- and postsynaptic spiking required for strengthening and weakening of the retinotectal synapse. Surprisingly, our results showed that correlation is not the only factor involved. The order of spiking was critical—repetitive spiking of the RGC within ~20 ms before the tectal cell spiking led to synaptic potentiation, but RGC spiking after the tectal cell spiking within a similar ~20 ms interval caused synapse depression (Zhang et al., 1998). Both potentiation and depression were N-methyl-D-aspartate (NMDA) receptor-dependent, in line with an earlier finding on the induction of LTP and long-term depression (LTD) in cortical slices (Markram et al., 1997). Within the context of synaptic competition *in vivo,* we characterized the complete time window of spike timing-dependent plasticity (STDP). In parallel experiments, Guo-qiang Bi also found similar STDP time windows at synapses between cultured hippocampal neurons (Bi and Poo, 1998). Within a few years, STDP was found by many labs in various types of excitatory and inhibitory synapses, with some variations in the time window and amplitude of LTP/LTD (Dan and Poo, 2006; Lu et al., 2007).

Backpropagation of LTP and LTD

Perhaps the most novel finding from my laboratory at La Jolla was the backpropagation of LTP and LTD from the output synapses of a neuron to input synapses on its dendrites and to other output synapses made by its axon collaterals. This discovery began in 1997 when two unrelated threads converged in my UCSD lab. The first was my memory of Crick's comment that the powerful "backpropagation" algorithm for training artificial neural networks is biologically implausible (Crick, 1989)—signals in real neurons propagate from dendrites to the axon, but not the other way around. The other thread was the development of the method for simultaneous whole-cell recordings from three or more interconnected neurons in hippocampal cell cultures. While searching for an interesting problem for using the multipatch recording method,

it occurred to me that we could test whether synaptic modifications induced between a pair of neurons could spread to other synapses in the network. Reiko Fitzsimonds and Hong-jun Song soon discovered an extensive retrograde spread of activity-induced LTD to other synapses onto or made by the presynaptic neuron (Fitzsimonds et al., 1997). Hui-zhong Tao and Li Zhang later showed that LTP could also backpropagate, and there was no forward propagation of LTD or LTP to the output synapses of the postsynaptic neuron (Tao et al., 2000). In retrospect, there was a prelude to these findings in nerve-muscle cultures, when Sydney Cash found that presynaptic depression caused by Ca^{2+} elevation in the postsynaptic myocyte could spread to other synapses made by the same presynaptic neuron on other myocytes (Cash et al., 1996).

Our findings on the presynaptic spread of LTP/LTD did not raise much interest in the plasticity field, probably because they were found in cell cultures. It took a few years before I realized that the best place to demonstrate this phenomenon *in vivo* is in the retinotectal system, where backpropagation of synaptic modifications from the tectum to retina could be attributed only to the spread of signals within the presynaptic neuron rather than feedback connections. A few years later, a postdoc fellow, Julin Du, was able to show that BDNF-induced potentiation and activity-induced LTP or LTD at retinotectal synapses could indeed backpropagate through the optic nerve to cause corresponding potentiation and depression of bipolar cell synapses on retinal ganglion cells (Du and Poo, 2004; Du et al., 2009). Nevertheless, the physiological significance of this phenomenon remains unclear. Having corresponding modifications of input synapses in accordance with those occurring at output synapses of a neuron may help to achieve more efficient activity-dependent refinement of neural circuits.

Kuffler Lectures

In 1998, I initiated the annual Kuffler lectures series at UCSD, using the endowment fund associated with the Kuffler Chair. Each year, a distinguished neuroscientist was invited to UCSD for a week, during which the lecturer gave a set of three lectures to summarize his or her life's work and interacted with neuroscience faculty and students via lab visits, luncheons, and dinners. The visit ended with a grand dinner with La Jolla neuroscience community in the Birch Aquarium at Scripps overlooking the Pacific Ocean. David Hubel, Francis Crick, and Sydney Brenner were the first three lecturers before I left La Jolla, and the Kuffler lectures continue to this day. The three lectures by Crick in 1999 were entitled: "Neuroscience: The Impact of Molecular Biology," "Consciousness: The Nature of the Problem," and "Consciousness: Recent Ideas and Experiments." I made a poster for his lectures using the M. C. Escher's lithograph *Bond of Union* (1956), in which two heads face each other, linked by two helical ribbons, with molecule-like spheres floating through them. I thought it symbolizes well Crick's transition from molecular

biology into the nature of consciousness. Crick must have liked the choice because he quickly accepted the poster design.

University of California at Berkeley

In 2000, I was persuaded to move my laboratory to Berkeley, where my close friend Yang Dan had set up her laboratory a few years earlier, and weekend travel became too difficult. A big attraction of the Berkeley community was the strong developmental neurobiology group consisting of David Bentley, Corey Goodman, Carla Shatz, John Ngai, Gunther Stent, and David Weisblat, and also the towering eucalyptus forest facing my office window. Joining the Berkeley faculty was also a belated consolation to my failure to get into Berkeley's biophysics graduate program 30 years earlier.

Functional Consequence of STDP

In the Berkeley lab, we continued to study the functional implications of STDP, which could potentially encode temporal sequence information. Florian Engert, Huizhong Tao, and Li Zhang showed that after conditioning with repetitive unidirectional moving stimuli, *Xenopus* optic tectum neurons developed long-lasting direction-selective responses to the same stimulus used in training (Engart et al., 2002), and this was due to STDP of retinotectal synapses induced by sequential firing of RGC inputs (Mu and Poo, 2006). Moving stimuli also induced sequence firing of rat V1 neurons, and repetitive training with a moving stimulus led to the storage of the memory of sequential firing that could be retrieved by activating only neurons that fired early in the sequence during training (Xu et al., 2012). Besides temporal sequence, STDP mechanism could also help to store interval information. Guo-qiang Bi showed that a network formed by cultured hippocampal neurons could store the memory of the time interval (up to 100 ms) between two sequential stimuli repetitively applied to a neuron in the circuit. This was achieved by STDP at various synaptic sites within polysynaptic pathways, based on differential delays in polysynaptic spike propagation to specific pre- and postsynaptic cells (Bi and Poo, 1999). Mechanisms for storing temporal information over seconds and minutes remains rather elusive. Reverberative neural activity within the network could serve to store temporal information over a period of seconds, as shown by German Sumbre in zebrafish larvae. Following repetitive visual stimuli at a regular interval (up to 10 s), the optic tectum exhibited reverberating activity waves at the same interval for a brief period (up to 30 s) (Sumbre et al., 2008).

Spaced vs. Mass Learning

The *Xenopus* retinotectal system also allowed us to address the role of spontaneous activity in the brain. In 2003, a postdoc fellow Qiang Zhou made a

serendipitous discovery. He normally used whole-cell voltage clamp recording of the tectal neuron to monitor the stability of LTP or LTD induced at retinotectal synapses by one episode of repetitive visual stimuli or by a train of theta-burst stimulation. One day somehow he switched the whole-cell recording mode from voltage-clamp to current-clamp, allowing the recorded neuron to undergo spontaneous spiking. To his surprise, normally very stable LTP and LTD found in continuous voltage-clamp mode decayed gradually within 30–60 minutes. This erasure of LTP/LTD by spontaneous activity depended on the activation of NMDA receptors, similar to that for LTP/LTD induction. However, this effect of spontaneous activity could be prevented if the single episode of repetitive stimuli was split into three episodes that were spaced a few minutes apart (while keeping the same total number of stimuli). Most interestingly, there was an optimal interval for the spaced stimulation (~5 min in this case); spacing either too short or too long was ineffective in stabilizing LTP/LTD (Zhou et al., 2003). The advantage of spaced over mass learning is well known in memory research. This finding offers a synaptic basis for determining the optimal interval for spaced learning; it also points to a potential function of spontaneous activity—clearance of memories that are not worth keeping in the brain.

Development and Plasticity of GABAergic Transmission

A group of free-spirited students and postdocs in my lab in La Jolla and Berkeley had ventured into many problems associated with early cortical development. On some occasions, I became aware of their work only after they came to talk to me about their findings. In a memorable day in La Jolla, Karunesh Ganguly and Alejandro Schinder surprised me with the result of a very simple experiment, in which they found that GABA added to the culture of cortical neurons could speed up the developmental switch of GABA's action from excitation to inhibition. They soon found that GABA-induced Ca^{2+} elevation in developing neurons could upregulate the expression of a K^+/Cl^- co-transporter KCC2, which reduces the excitatory action of GABA by extrusion of cytoplasmic Cl^- (Ganguly et al., 2001).

After developmental maturation, inhibitory GABA synapses are susceptible to further activity-dependent modifications. Two postdoctoral fellows, Hubert Fiumelli and Laura Cancedda, found that repetitive postsynaptic spiking could induce LTD of GABAergic synapses, due to a Ca^{2+}-dependent downregulation of KCC2 function (Fiumelli et al., 2005). High-frequency correlated pre- and postsynaptic spiking also induced LTP of inhibitory synapses, through $GABA_B$ receptor-mediated local upregulation of $Na^+/K^+/2Cl^-$ cotransporter (NKCC1) activity that increases cytoplasmic Cl^- (Xu et al., 2008). Unlike local mechanisms underlying plasticity of glutamatergic synapses that involve mostly modifications of presynaptic transmitter secretion and/or postsynaptic transmitter receptors, transporter modulation will affect cytoplasmic Cl^- concentration over the dendritic domain beyond

individual synapses. Thus, LTP/LTD induced at an inhibitory synapse could spread substantially to other inhibitory synapses along the dendrite.

Development and Maintenance of Neuronal Polarity

My interest in polarity formation in developing neurons was revived in 2008 when Maya Shelly arrived in Berkeley for her postdoctoral research. Maya proposed to study the molecular basis of axon/dendrite polarization in developing neurons, particularly the function of LKB1/STRAD complex that plays a crucial role in epithelia polarization, a subject of her doctoral studies. Using Gary Banker's model system of isolated hippocampal neurons in culture (Banker and Cowan, 1977), Maya showed that LKB1 phosphorylation at the tip of a neurite represents a molecular determinant for axon initiation (Shelly et al., 2007). Such an event could be initiated by local exposure of the neurite to extracellular BDNF, which activates PKA by local elevation of the cAMP level in the neuron.

The mechanism of axon/dendrite differentiation must account for the Gary Banker's observation that each cultured hippocampal neuron initially develops multiple neurites, but only one differentiates into the axon and the rest become dendrites. In 1951, Alan Turing proposed a molecular mechanism of morphogenesis in which polarized biological structure could emerge through a slow-diffusing local autocatalytic activator coupled with the generation of a fast-diffusing global inhibitor. This proposal has inspired many biological studies on the molecular mechanism underlying the polarization of cells and tissues. Our venture into the Turing mechanism began with the discovery that cAMP and cGMP, two second messenger molecules known to exert reciprocal antagonistic regulation, are involved in neuronal polarization. Sarah Heilshorn, who joined the lab to learn neurobiology before taking her faculty position at Stanford, devised a neat culture substrate with bound stripes of chemicals. Together with Maya Shelly and Byungkoon Lim, she found that locally elevated cAMP and cGMP activities in the hippocampal neurite promote its preferential differentiation into axon and dendrite, respectively (Shelly et al., 2010). Further experiments suggest that the local autocatalytic process at the growing axon could begin with a stochastic fluctuation of local cAMP or localized presence of extracellular factors (such as BDNF) that elevates cytoplasmic cAMP. This cAMP elevation could induce localized BDNF secretion that caused local TrkB activation through autocrine action of BDNF, and TrkB activation in turn further elevated local cAMP level, promoting more local BDNF secretion (Cheng et al., 2011). Such autocatalytic process eventually leads to stable cAMP accumulation locally and axon differentiation via activation of LKB1 and other axon determinants. Conversely, because of reciprocal suppression between cAMP and cGMP, cGMP elevation gradually develops at distant neurites, promoting their differentiation into dendrites (Cheng and Poo, 2012).

After axon/dendrite differentiation, the maintenance of the polarity becomes the issue—how cytoplasmic and membrane components are shipped and maintained in the axon vs. dendrite compartments. While at my Columbia lab, Bettina Winckler first began to study the existence of a diffusion barrier for membrane components at the axon hillock (Winckler and Poo, 1996). She found no apparent barrier for lipids, but her later work in Ira Mellman's laboratory at Yale showed a clear diffusion barrier for membrane proteins (Winckler et al., 1999). In the cytoplasm, there is also the need for some cellular structure that regulates the molecular traffic in and out of the axon. Ai-hong Song in my Shanghai lab later found the emergence of a cytoplasmic "filter" at the hillock after axon differentiation—an actin-based meshwork that impedes free diffusion of large macromolecules, but permits the passage of specific kinesin-driven transport of axon-destined cellular cargos (Song et al., 2009). This line of work on axon/dendrite polarization and maintenance in my lab ended as the lab members involved began to set up their own laboratories to continue the work.

An Infamous E-mail Letter

In 2001, my Berkeley lab had grown to a size that was becoming difficult to supervise. While being disturbed by the lack of progress and frequent absence of some people in the lab, I wrote an e-mail to all lab members, suggesting that they should spend sufficient time in the lab (at least eight hours a day and six days a week) and inform me of their absences from the lab for one day or more. I made a strong argument that continuous concentration in lab work was necessary to achieve significant progress, and I asked those who disagreed with my "rules" to leave the lab. One student (out of more than 20 people) indeed left. This e-mail was later widely circulated on the web, and I was accused by some of being a "dictator" Interestingly, I was told that my e-email was found on the wall of many labs, but whether it was to endorse or condemn my rules was unclear. Some Chinese websites posted versions of my letter with added rules (presumably by some supervisors), such as no after-lunch nap in the lab, a habit of many Chinese students. In a report on my work in China (Cyranoski, 2011), I was quizzed on this letter and on my "dictatorial" style in the lab. The best answers were in fact provided by some of my formal lab members (now exceeding more than 150), who have since established laboratories in five continents.

Institute of Neuroscience, Shanghai

The Chinese Academy of Sciences (CAS) went through a revitalization process in the late 1990s, through the effort of its then President Yong-Xiang Lu, who secured a substantial amount of government funding for CAS institutes. In the spring of 1998, I met President Lu in a café at the San Francisco

Airport, the first stop of a CAS delegation to the United States. Lu convinced me that the time was ripe for CAS to revamp its neuroscience program, and he invited me to visit several CAS institutes and make recommendations. In the summer of 1998, I visited Shanghai Institute of Brain Research (SIBR), the Institute of Physiology in Shanghai, and the Institute of Biophysics in Beijing. I found that the conditions for research were quite depressing. For more than a decade, China's open-door policy had led to a massive exodus of scientists to institutions overseas, leaving these CAS institutes with very few active investigators. There were only three labs left in SIBR, which was founded by Hsiang-Tong Chang, the "grandfather" of modern Chinese brain research. While struggling through my report to CAS, I decided that the best approach to uplift Chinese neuroscience was to establish a new institute, with new infrastructure and an attractive environment for recruitment. I asked the then-SIBR director Chien-Ping Wu and three colleagues in the United States (Bai Lu, Lin Mei, and Yi Rao, who were then running a joint lab at SIBR) to cosponsor the proposal for founding the Institute of Neuroscience (ION). The proposal was quickly approved by President Lu, with the condition that I serve as its director. During the first decade of my directorship, I made regular bimonthly trips to Shanghai, each lasting one to two weeks. Because I was still a full-time faculty and running an active lab in Berkeley, the ION directorship was considered to be part of my uncompensated academic activity, reported annually to the Berkeley administration. By early 2012, however, frequent trips became too exhausting, and I began to phase out my Berkeley lab, and formally retired from Berkeley in 2014. My ION directorship ended by 2019, but I continued to serve an advisory role as the scientific director and run a laboratory on neural plasticity. Over the two decades of my directorship, I was fortunate to have had the help of a most dedicated and trustworthy manager, Yan Wang, who was able to deal with most administrative affairs, allowing me to focus mainly on academic matters.

At the beginning of ION, six lab heads were recruited from within China. To justify the salary and research support at a level much higher than that in other Chinese institutions, each laboratory was required to publish at least one paper every four years in the "top seven" international neuroscience journals. Many Chinese colleagues thought that was impossible because there had not been a single paper published in those journals over the previous decade based on work done in China. However, it turned out that every lab met the requirement. By 2005, it was estimated that ION investigators were publishing about one-third of all high-profile papers in biological sciences from China. With this publication record, the lab head recruitment became much easier, and ION grew rapidly. New to the CAS system, I introduced in 2002 biennial lab reviews by an international scientific advisory board, which provided critical comments on research programs and conducted tenure reviews for ION investigators. No one was exempted from this review. This requirement created a crisis in 2005, when one senior

ION member refused to be reviewed because he was already elected as a CAS academician. I stood firm on this issue. CAS leadership had to intervene and resolve the crisis by offering him a very nice position elsewhere within the CAS system. Another difficult event was the case of scientific misconduct in 2009. An investigation committee consisting of both external and internal scientists found clear evidence of data falsification by a lab head. I therefore decided to terminate the ION appointment of this individual. This was a step rarely taken by a Chinese institution, and it created quite a commotion in the neuroscience community.

Setting up a rigorous graduate training program was one of my top priorities at ION. Many standard practices in U.S. universities were adopted, such as laboratory rotations before the selection of thesis adviser, annual thesis progress reports to a committee, and interaction with seminar speakers at lunch meetings. Perhaps the most notable practice, enforced through my personal participation in more than 100 thesis committee meetings over the years, was the uninhibited critique of the students' research projects in the presence of their thesis advisers. This practice allowed the student to truly benefit from the input of multiple faculty members. The annual meetings of ION, where students and postdocs from each lab make oral presentations, also involved active and provocative exchanges. This was quite a cultural change for a Chinese institution where face-saving is very important. I gave a director's address in each annual meeting, on various aspects of scientific research and graduate training, and transcripts of my talks were widely circulated on the Chinese internet.

Over the past two decades, 23 lab heads left the ION for other institutions in China, many of them taking leadership positions. Among them were five CAS academicians who were elected based on their research achievements in ION. These scientists left with their graduate students, postdoctoral fellows, external funding, and equipment. I made very little effort to retain them or to set barriers to prevent their move, and I was blamed by the CAS administration for being unable to keep excellent scientists and maintain the "stability" of ION. My own experience taught me that mobility is a good thing for an institution and the scientific community. Indeed, most of the people who left ION excelled in their careers and introduced many good ION practices to their new institutions. In any case, ION has accomplished its goal of being an institute that nurtures young neuroscientists in China.

I also run a laboratory of neural plasticity in ION with a cosupervisor, Shumin Duan (1999–2005) and Xiaohui Zhang (2006–2012). Under this arrangement, the laboratory was very productive and many graduate students carried out doctoral research on a variety of subjects in axon guidance, neuronal polarization and migration, synaptic plasticity, neuron-glia interactions, and cognitive functions. Most students went abroad for postdoctoral training afterward, and many are now running their own labs in China or abroad.

STDP beyond Synapses

The neural plasticity field is mostly concerned with plasticity of the synapse. In 2000, a MS/Ph.D student, Karunesh Ganguly, in my UCSD lab found that LTP induced at the synapse between cultured hippocampal neurons was accompanied by increased intrinsic excitability of the presynaptic neuron, because of a PKC-dependent modification of presynaptic Na^+ channels (Ganguly et al., 2000). Four years later, Chengyu Li and Jiang-teng Lu in my Shanghai lab showed that the excitability of presynaptic neurons was also reduced after LTD induction in both hippocampal cultures and slices, an effect requiring presynaptic PKA- and PKC-dependent modification of slow-inactivating K^+ channels (Li et al., 2004). The presynaptic effects on intrinsic neuronal excitability were observed at the soma, indicating global backpropagation of signals from the presynaptic nerve terminals to the entire presynaptic neuron. Using hippocampal slices, Zhiru Wang and Ninglong Xu measured the spatial summation of synaptic inputs at the dendrites of CA1 neurons and found that LTP and LTD induction also results in a persistent increase and decrease, respectively, in the linearity of dendritic summation of synaptic potentials. These changes of summation properties were specific to the modified inputs and reflect localized dendritic changes involving I_h channels and NMDA receptors (Wang et al., 2003). Thus, STDP involves not only local LTP/LTD of synaptic efficacy but also substantial changes beyond synapses in both pre- and postsynaptic neurons. Notably, postsynaptic spread of the dendritic changes associated with LTP/LTD is much more restricted than the presynaptic effects, consistent with our previous observation that there is extensive backpropagation but no forward propagation of LTP/LTD.

Nonhuman Primate Research.

Ten years after founding ION, I became increasingly aware of the need for using nonhuman primates (NHPs) to study higher cognitive functions and brain disorders. Using a director's discretionary fund provided by CAS, I established facilities for rearing macaque and marmoset monkeys in 2009 and began to recruit investigators interested in studying NHPs. As of 2023, about one-third of ION's 55 labs were doing NHP-related research. In 2018, Nikos Logothetis visited Shanghai, and we discussed the possibility of setting up an International Center for Primate Brain Research (ICPBR) in Shanghai, with the goal of establishing NHP facilities with high international standards and open to investigators around the world to conduct NHP research. With a special fund from the Shanghai government, ICPBR became a reality in 2020, when Nikos and four other laboratory heads moved from Tubingen to Shanghai. Beginning in 2018, ION also began a collaboration with the International Brain Research Organization (IBRO) to sponsor

a regular Summer School in Primate Neurobiology, with the goal of promoting an interest among young researchers in studying NHPs.

A strong justification for the use of NHPs is the potential development of animal models of brain diseases, given the obvious inadequacy of mouse models of brain disorders, especially psychiatry diseases. In 2016, a graduate student, Zhen Liu, in Zilong Qiu's ION laboratory, working with the macaque platform director, Qiang Sun, overexpressed the human MECP2 gene in 1-cell monkey zygotes obtained via *in vitro* fertilization, implanted the embryos in surrogate female monkeys, and generated monkey offspring. These monkeys showed Rett syndrome–like phenotypes, such as repetitive movements and deficits in social interactions (Liu et al., 2016). A few years later, Hong-jun Chang and Qiang Sun generated macaque monkeys with the deletion of a circadian core gene BMAL1 using CRISPR-Cas9 editing in 1-cell zygotes. The monkeys exhibited sleep disorders, dampened circadian oscillations of circulating hormones, and a variety of psychotic phenotypes, including anxiety and depression-like behaviors and defective social interactions (Qiu et al., 2019). A variety of macaque models for brain disorders with strong genetic contributions, including Parkinson's disease, fragile-X syndromes, and Angelman's syndrome, are now being constructed with this gene-editing approach at the ION facility.

Cloning of Macaque Monkeys

The gene-editing approach has generated monkeys with varying degrees of disease phenotypes, because of variations in the genetic background of edited embryos. Mouse models with homogenous genetic background had been produced by inbreeding over many generations, but this is not practical for the macaque, which has a long generation time of five to six years. Cloning of animals by the somatic cell nucleus transfer (SCNT) method appears to be a solution. Since the birth of Dolly the Sheep in 1996, cloning by SCNT has been achieved for more than a dozen mammalian species, but not in NHPs. In November 2012, I called a small meeting among ION researchers in the suburban town of Wuzhen, and put forward the cloning of macaque monkeys as a major goal of ION's NHP platform. By improving the SCNT protocol and screening epigenetic modulators that could reactivate development-relevant genes in the transplanted somatic nucleus, Zhen Liu (by then a postdoctoral fellow in my lab) and Qiang Sun succeeded in cloning macaque monkeys by SCNT in late 2017, using cultured fibroblasts derived from an aborted female fetus (Liu et al., 2018). I named these two macaque sisters Zhong-zhong and Hua-hua (with "Zhong hua" meaning Chinese). They now live well among a group of monkeys in our facility. The SCNT method was soon used to generate a group of five cloned monkeys from the skin fibroblasts of a donor BMAL1-deleted monkey that exhibited the most severe psychotic phenotypes (Liu et al., 2019). The cloned monkeys

showed similar anxiety and depression-like phenotypes as the donor monkey. Hong-Jun Chang and his colleagues are now using these BMAL1-deleted monkeys for preclinical efficacy tests of new candidate drugs for anxiety and depression.

Studying Higher Cognitive Functions in NHPs

A well-recognized use of NHPs is to study higher cognitive functions that are unique to primates. For many years, primatologists have shown that some great apes, but not macaque monkeys, exhibit self-consciousness by passing the mirror self-recognition test—touching a dye mark on the face when seeing his or her own face in a mirror. In 2014, Neng Gong and I decided to train monkeys to perform the mirror self-recognition task. We projected a laser light spot on a board behind the head of a head-fixed monkey, and the monkey needed to touch the spot seen in the mirror to receive a reward. By this training, the monkey learned the association between the mirror image of the arm and hand with his own proprioceptive sensation. After a few weeks, all five trained monkeys realized that the mirror image represented their own body and were able to pass the face mark test of mirror self-recognition (Chang et al., 2017). Thus, mirror self-recognition represents the animal's ability to associate the mirror image with his own body. The loss of mirror self-recognition ability found in severe cases of mental disorders, such as Alzheimer disease, schizophrenia, and autism, probably reflects defective multisensory association and recall, and our training protocol for monkeys may prove to be useful as a therapeutic tool. In another study using NHPs, Huan-huan Zeng in my lab used two-photon Ca^{2+} imaging to monitor the activity of a large population of neurons in the primary auditory cortex of awake marmosets, which have a complex repertoire of calls. He discovered many types of neurons that responded selectively to different calls in the primary auditory cortex, and these cell-selective cells were largely distinct from the well-known frequency-tuned cells in the primary auditory cortex (Zeng et al., 2022). This is a first step in studying neural processing of call sequences in the marmoset brain.

China Brain Project

When the global wave of big brain projects began in 2013, I was asked by the China's Ministry of Science and Technology to organize a series of meetings to discuss and plan a national brain project. When it formally began in 2021, China Brain Project (CBP) represented a 10-year program of basic research on the neural basis of brain functions and on applied neuroscience that includes diagnosis and intervention of major brain diseases and brain-machine intelligence technology, such as brain-computer interface,

neuromodulation methods, and brain-inspired machine learning and computing devices (Poo et al., 2016). The basic neuroscience program includes a component of mesoscopic brain mapping—to identify all cell types in the mouse, monkey, and human brains, and to map the input/output connectivity of each neuron type in all brain areas of mice and monkeys. Since the U.S. BRAIN Initiative has made much progress in mapping cell types and connectivity in the mouse brain, I thought CBP could make more substantial contributions by focusing on the macaque brain (Poo, 2022), which is much more complex and requires large-scale collaborative efforts around the globe. This is exemplified by the recent mapping of spatial transcriptomes of the entire macaque cortex at the single-cell resolution (Chen et al., 2023). It identifies layer- and region-specific putative cell subtypes and paves the way for mapping cell type-specific connectomes of the monkey brain. Single-cell spatial transcriptomes also provide information on the neighborhood composition of various cell types in the brain. With further information on the temporal changes and cross-species differences in gene expression, one can now explore the molecular and cellular mechanisms underlying morphogenesis and evolution of the brain.

Brain-Inspired Machine Learning

Our discovery that LTP/LTD could backpropagate in the presynaptic neuron was inspired by the backpropagation algorithm of artificial neural networks (ANNs). I had deliberately termed the phenomenon "backpropagation," but it failed to attract the attention of ANN community. Nearly two decades later, I convinced Bo Xu and his colleagues at the Institute of Automation of Chinese Academy of Sciences in Beijing to incorporate this natural backpropagation as part of a learning algorithm for spiking neural network (SNN). They used STDP-based LTP/LTD to induce modification of synapses on output-layer neurons of a three-layer SNN, based on the timing between network-induced spiking of the output neuron and expected spiking, which serves as the supervising signal. The resultant potentiation/depression of output-layer synapses was allowed to backpropagate to a subset of synapses on the hidden-layer neurons. This learning rule enabled the SNN to outperform other conventional backpropagation-based algorithms in learning several benchmark tasks in both accuracy and computational cost (Zhang et al., 2021). Furthermore, inspired by the finding on neuromodulator-induced STDP rules, they implemented a metaplasticity mechanism in the SNN learning algorithm, resulting in a greatly reduced catastrophic erasure of previous memories in several standard continuous learning tasks as well as a reduced computational cost (Zhang et al., 2023). Brain-inspired machine learning methods are likely to contribute to the future development of energy-efficient artificial intelligence.

Retrospective and Prospective Research

For students and postdoctoral fellows who joined my laboratory with a limited biology background, I often suggest that they read a good textbook, identify a hypothesis of interest, repeat the experiments that had laid the foundation for the hypothesis by using newly developed methodologies. A good example was Sergei Popov, a physicist from Moscow, who reexamined the "tip growth hypothesis" of growth cone extension as described in the first edition of *Molecular Biology of the Cell* by Alberts and colleagues. The hypothesis states that new membrane materials for axon growth are transported in the cytoplasm to the growth cone, followed by local membrane insertion. The main evidence is that carbon particles attached to the membrane of growing neurites did not move forward. Instead of using carbon particles, Popov loaded a small amount of fluorescent lipids locally into the axon of cultured neurons and monitored the profile of fluorescent lipids with time. He found that, as the fluorescent lipid patch spread laterally in the membrane, the center of mass of the fluorescent patch moved forward in the growth cone. This approach suggested that new membrane is inserted at the base of the axon, pushing a bulk membrane flow toward the tip (Popov et al., 1993). However, Popov later showed in his own lab that forward membrane flow occurs only in fast-growing axons, whereas tip insertion is favored when the growth rate is low. The new problem is to find out the cytoplasmic mechanism that regulates the site of new membrane insertion. In any case, the tip growth hypothesis was removed in later editions of the textbook by Alberts and colleagues.

Our experiments on the developing retinotectal system also aimed initially at reexamining the textbook version of Hebb's hypothesis on synaptic modification by correlated activity. Using a newly developed *in vivo* whole-cell recording, we found results that led to the discovery of time windows for STDP and the revision of Hebb's hypothesis (Bi and Poo, 2001). I call this way of doing science the "retrospective" approach, and its outcome is always useful—either confirmation and strengthening of a textbook hypothesis or, more rewardingly, its refutation or revision. This is distinct from the normal "prospective" approach—designing new experiments to test an existing hypothesis, and revising the hypothesis when sufficient refuting evidence has been accumulated. Although both approaches could achieve the same goal of revising existing hypotheses, the retrospective approach seems more effective, given that it aims directly at its experimental foundation.

Epilogue

I was invited back to La Jolla to give the 20th Kuffler lectures in 2019. I chose the title of "Pathfinding in Neurobiology" to describe my scientific ventures in three areas: neuronal polarization and axon guidance, synapse formation

and plasticity, and memory and cognition. This title appeared more purposeful than the one I used for my 2016 Gruber Prize lecture: "Random Walks in Neurobiology." I felt that my scientific career was a pathfinding process largely driven by circumstances and serendipity. In writing this autobiography, I noted the apparent haphazardness of many choices along my career path and research problems, and I reflected on the fact that many research problems were not pursued in sufficient depth before I moved onto others. I could attribute this to the lack of formal training in a specific area of biology that could have consolidated a long-term goal of solving a specific biological problem. It may also have been my lack of patience in pursuing the nuances of a particular subject. Many of my research ventures were also initiated by students and postdocs who happened to join my lab; some of them have made substantial progress in pursuing the subject later in their own labs.

In retrospect, the most enjoyable period of my scientific life was the time I had weekly individual meetings with each lab member in my office, when I learned from what they had learned and figured out with them the most interesting experiment to do. Arriving at a good plan for experiments was as exciting and rewarding to me as seeing experimental results. For this, extensive conversational discourse is essential. Gunther Stent once asked over the dinner table, "If you had unlimited resource[s] to pursue your interest alone on a desolate island and are forbidden from talking with anyone, would you do it?" Indeed, my scientific pathfinding was accompanied and largely driven by people I had the fortune to interact closely with. To them, I owe an enormous gratitude. Some unusual circumstances I encountered and took advantages of, as someone with cultural background in both the East and West, have led to many ventures beyond the confines of my own laboratory. Besides being a researcher, I have also enjoyed my life as a teacher and administrator and, as such, I probably have left more lasting footprints.

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