The History of Neuroscience in Autobiography
Volume 5

Edited by Larry R. Squire
Published by Society for Neuroscience
ISBN: 0-12-370514-2

Lynn T. Landmesser

pp. 382–411

https://doi.org/10.1016/S1874-6055(06)80032-0

Lynn T. Landmesser

Born:

Santa Ana, California
November 30, 1943

Education:

University of California Los Angeles, B.A. (Zoology, 1965)
University of California Los Angeles, Ph.D. (Zoology, 1969)

Appointments:

Postdoctoral Fellow, University of Utah (1969)
Yale University (1972)
University of Connecticut (1983)
Case Western Reserve University (1993)
Chair, Department of Neurosciences, Case Western Reserve University (1999)

Honors and Awards (Selected):

President, Society for Developmental Biology (1988–1989)
Arturo Rosenblueth Distinguished Professor, Center for Advanced Studies, IPN Mexico (1987)
Wiersma Visiting Professor of Neuroscience, California Institute of Technology (1989)
Secretary, Society for Neuroscience (1992–1994)
American Academy of Arts and Sciences (1993)
Arline H. and Curtis F. Garvin Professor of Medicine (2001)
National Academy of Sciences, U.S.A. (2001)

Lynn Landmesser utilized the embryonic chick spinal cord to provide the first evidence for early specification of motoneuron subtypes and their selective pathfinding. Her laboratory also demonstrated motoneuron pool-specific spontaneous bursting patterns and characterized the circuits that drive the earliest rhythmic activity and defined its role in motor circuit formation. She has also elucidated the roles of NCAM and polysialic acid in neuromuscular development, synaptic maturation, and transmission.

Lynn T. Landmesser

But for the Second World War, I would have grown up around East Orange, New Jersey, where the families of my father Charles Landmesser and my mother Eleanor Cerveny Landmesser had settled after their parents had emigrated from Germany/Ireland and Czechoslovakia, respectively, a generation before. My father, who fought in the Pacific during the war, was stationed at Camp Pendleton Marine Base in California and thus in 1943 I came to be born in Santa Ana and spent the first year of my life in Laguna Beach, often literally on the beach as my parents rented a house only a stone's throw from the Pacific Ocean. Loving the physical beauty of California and the possibility for change, my parents decided to settle there when the war ended. With no offense intended toward New Jersey, I will always be grateful for this chance of fate that allowed me to grow up in California, for I am certain that this shaped in important ways my decision to become a biological scientist. First, by exploring, as a child, the diverse environments of California, often on camping vacations, I became fascinated by the diversity of life and the adaptation of individual species to their environments. This later motivated me toward trying to understand how the behavior of such species, which was in the end the product of their nervous systems, could be so exquisitely tailored to their individual environments. Second, the State of California, with incredible foresight, had decided to create a first-class university system that was the equal of the Ivy League but which was available to all its citizens, regardless of their means.

I have always felt that had I grown up on the East Coast, my family being of modest means, I was likely not to have attended college. I probably would have done something interesting and creative with my life, but I am almost certain that I would not have become a neuroscientist. In looking back over my life, still not believing that I am at that stage to be doing so, I was struck by how the path that I eventually followed was shaped by so many chance occurrences. I suppose all lives are shaped by such chances, but I hope that by conveying these reminiscences I can reassure those that are striving toward or even just beginning to dream about a career in science that the same place can be reached by many different paths and that there is not one true correct route that must be followed.

Early Years

My father's interest in nature and the environment had a huge impact on my decision to become a biological scientist. Although my mother was always very supportive, camping vacations were something she bravely endured for the sake of the family. Whether we were exploring tide pools near Torrey Pines, watching the moonlight on the sands of the Anza Borrego desert, or hiking in mountain meadows near Sequoia my father helped me appreciate the extraordinary diversity of animal species and their adaptive behavior. These experiences no doubt contributed to my goal, articulated in an essay that we eighth graders at St. Patrick's elementary school in San Diego were asked to write as graduation approached, and which was decidedly atypical for a female at that time, to become a diver for Scripps Institute of Oceanography. By the time I graduated from high school at Our Lady of Peace Academy in 1961, with my sights on UCLA, I had decided on the less exciting but more employable career of a medical technologist. It seemed that this UCLA program would let me combine my interest in biology with a marketable skill as I sought employment 4 years hence. It was clear that after sacrificing to send me to college for 4 years, my family expected me to be able to support myself upon graduation.

UCLA and My Discovery of Neuroscience

I arrived at UCLA, which then had a student body of 40,000 or so, feeling both excitement and a bit of trepidation. It is often said that females do better at smaller private schools where they receive more personal attention, but for me the anonymity of such a large campus was comforting, for it allowed me the possibility to explore and make mistakes unnoticed and to make my own decisions, wise or not. It was to be my home for the next 8 years, during which I experienced and participated in the social and political upheavals that marked the 1960s. It was an exciting place both academically and socially with the civil rights movement and the opposition to the gradually escalating war in Vietnam having a lasting impact on my fellow students and me. Fortunately, the interesting and well-taught general biology course I was required to take my first semester caused me to abandon the medical technologist program for a major in biology. I postponed worrying about how I would obtain a job with a biology major until graduation. I was not interested in medicine, as most of my fellow biology majors were, and I had not yet realized that one could have a career doing science in academia. I did well academically especially enjoying physiology, but by my junior year I was beginning to be bored by simply acquiring new information in courses. Also from the beginning of my junior year I had begun to support myself by working 20 hours per week in the geology department, extracting and classifying fossils from rock samples.

Although this provided me with the confidence that I could indeed support myself, financial concerns together with anguish over the social and moral problems facing our country, including racism, poverty, and the war in Vietnam, made much of the course work seem esoteric and irrelevant to real life. Returning to San Diego for the summer, I had almost decided on dropping out of UCLA for a few years. However, I had greatly enjoyed carrying out an independent research project on crab behavior at the end of an invertebrate zoology course and wrote to that professor to see if I could carry out an independent research program equivalent to several courses that UCLA offered to senior zoology majors. Because he was on sabbatical, my letter was forwarded to Theodore (Ted) Bullock, a world-renown neuroscientist. He wrote back saying that, if I really wanted to explore the biological basis of behavior as I had indicated, I should take his neuroscience course in the fall and, doing well in that, I could do the research project in the spring. Had he not responded and I had left school to work for several years, I probably would not have become a neuroscientist. Thus, I will be forever grateful to him.

Happily I did well in the neuroscience course, which, like Ted's interests, was broad ranging from comparative neuroscience to membrane biophysics, vertebrate neuroanatomy, and behavior. He did not so much teach as point out interesting enigmas and problems that encouraged us to learn on our own. My independent research project involved determining the effect of temperature changes on the bursting properties of spontaneously active cells in the buccal ganglion of *Aplysia,* and I fondly remember collecting *Aplysia* with my teaching assistant Mike Mote from boat slips at the Marina del Rey. My fascination with behavior of the organism was quickly transferred to the behavior of cells, as my intracellular recordings revealed the individually unique bursting patterns of different identified cells. Alan Grinnell, a newly hired assistant professor, who later became my Ph.D. mentor, helped me with fabricating electrodes and I quickly fell in love with neuroscience.

I applied (rather late but was still accepted) to do a master's with Ted Bullock. However, having just been recruited to the new UCSD campus and planning to be away for extended periods the following year, he thought it wise for me to have Alan Grinnell as an on-site co-mentor. Because Ted was moving with a group of more senior graduate students and postdoctoral fellows and his lab renovations were not on schedule, I had come to the conclusion by the end of that first year that I would have more personal attention and probably make better progress if I remained at UCLA with Alan as my Ph.D. mentor. I had in the meantime set my sights higher than a master's degree.

Alan, who had interests in both bat echolocation from his Ph.D. work at Harvard and in spinal cord and neuromuscular physiology as a result of postdoctoral studies at University College London, generously provided

me with the intellectual freedom and support to pursue whatever I found most interesting. With tools being quite limited, progress in neuroscience those days required finding just the right experimentally tractable preparation to answer a specific question. Having toyed with several projects on cockroaches and moths, I settled on the crayfish muscle stretch receptor neuron as an excellent preparation to determine if transmitter receptors on neurons were selectively clustered at synaptic sites, as they had recently been shown to be at the neuromuscular junction. These large neurons were innervated by discrete bouton synapses on the soma and dendrites, and although lacking the antibodies and fluorescently tagged receptor antagonists we have today, it was possible to map the receptor distribution by iontophoresing transmitter locally. After several frustrating months of trying to get long-term stable recordings from the cells that this effort required, Susumu Hagiwara, a fine scientist and wonderful human being, who later became a member of my dissertation committee, informed me that such recordings were virtually impossible in the *Procambarus* species we had been using, and which I confess my fellow students and I used to filch from a pond at the Self Realization Fellowship complex near Sunset Boulevard. After ordering the preferred *Astacus* species from Oregon, I made rapid progress and was beginning to acquire data supporting receptor clustering when we were informed that due to severe flooding in Oregon no more crayfish would be available for 5–6 months.

Thus, it was again a chance of fate that caused me to turn my attention to an area that was to become my lifelong interest. Much of neuroscience research at that time was directed toward defining the connectivity of circuits and understanding membrane properties. The question of how such circuits were formed and either maintained or altered by experience during the life of an individual was largely ignored. Thanks to the incredible collections at the UCLA Biomedical Library, I was able to discover the two volume series of Cajal, published as the 1952 edition in French, which fortunately I was able to read. I was captivated by his lively and imaginative descriptions of growth cones battering their way around obstacles to reach their targets and how spinal cord circuits became assembled. Equally fascinating, and entertaining too, was his autobiography, *Recollections of My Life,* which proved one could begin a scientific career at the age of 40 and still excel, provided one was long-lived. However, lacking the tools at that time to probe underlying mechanisms, I decided to postpone the study of embryonic development.

Instead, long-term trophic interactions between neurons and their targets became my focus. A 1951 paper by De Castro, also in French and likely unavailable at many biomedical libraries, described some intriguing surgical cross-innervations in the cat that suggested that the central processes of sensory vagal neurons could make functional synapses with sympathetic ganglion cells in the superior cervical ganglion. Thus, inflating

a balloon in the stomach caused sympathetic reflexes such as dilation of the pupil. This suggested to me a way to determine the extent to which the properties of a given synapse were autonomous to the pre- or postsynaptic element and the extent to which the properties could be altered by one partner influencing the other. I decided to transplant the frog sartorius muscle, the vertebrate synapse where many properties of transmission had been described by Bernard Katz, Ricardo Miledi, and their colleagues at University College London, by suturing its tendons to the lower jaw and sternum, where it could be innervated by preganglionic cholinergic fibers in the gastric branch of the vagus nerve. Lack of surgical expertise did not keep me from plunging ahead but luckily the frogs were tough and recovered rapidly from the surgery and functional synapses formed.

Although the presynaptic properties of this hybrid synapse remained preganglionic, these autonomic axons were unable to induce junctional folds or the deposition of AChE postsynaptically. More intriguing, they appeared to have altered the pharmacological properties of the postsynaptic ACh receptors to become more ganglionic in nature. Today one would think of perhaps alterations in nicotinic receptor subunit expression and I have occasionally toyed with the idea of repeating these experiments with the modern tools now available. However, at that time such molecular changes could only be inferred by pharmacology.

Wishing to pursue these ideas further in postdoctoral studies, I was frustrated by the lack of scientists in the United States who were studying trophic interactions with modern electrophysiological approaches. However, a strong group in Prague was doing just this and one of them, Radan Beranek, had visited our laboratory and encouraged me to visit Prague on a planned trip to Europe the following summer. Steeped in cold war propaganda, it was with considerable trepidation that my brother Anton and I visited both Hungary and Czechoslovakia in the summer of 1967. We were surprised to find the people of both countries warm and outgoing and I was captivated by the almost magical charm of Prague. My decision made, I enrolled in a Czech course at UCLA and began preparations for a move abroad. Then, in August 1968 the Russians invaded! However, I was assured by my Czech colleagues that the presence of an American postdoctoral fellow there would not cause them difficulties, and I applied for and, amazingly for a time at the height of the cold war, received a National Institute of Health (NIH) postdoctoral award to pursue these studies.

Then, several months before I was to defend my dissertation, I received word that my proposed mentor, Radan Beranek, had died unexpectedly. This threw me into complete confusion. Alan Grinnell was away for several months on an expedition to New Guinea and essentially out of contact. NIH informed me that I would be allowed to transfer my fellowship to any reasonable laboratory in the United States, and Richard Orkand, a member of my dissertation committee, put me in touch with Ed Kravitz

at the neurobiology department at Harvard, who agreed to sponsor my postdoctoral studies. I am sure most readers will be incredulous, but although excited to study at what was considered the top neuroscience group in the country, my west coast prejudices made a move to Boston quite unthinkable. During our many enjoyable weekend outings to Boston during the two decades I came to spend in Connecticut, I would laugh at myself, but my feelings at that time were strong and real. The east seemed a bastion of old money and privilege, exactly what my compatriots and I, who came of age in the late 1960s had been idealistically struggling against.

Salt Lake City and My Foray into Developmental Neuroscience

As I respectfully disengaged from my commitment to go to Harvard, an alternative that made a lot of sense scientifically, if not politically, emerged. The physiology department at the University of Utah had also developed a strong presence in neuroscience under the leadership of Carleton (Cuy) Hunt. Guillermo Pilar, together with Bob Martin, had carried out, several years earlier, seminal studies on neuronal synaptic transmission in vertebrates, using the chick ciliary ganglion as a model preparation and he had expressed interest at a Western Nerve Net meeting (a predecessor of the Society for Neuroscience) in using the ciliary ganglion to study questions of specificity during regeneration and development. He enthusiastically welcomed me to join him in investigating this new area and I have never regretted this decision as it enabled me to explore a totally new discipline, uncluttered at that time by either concepts or facts and which later emerged as developmental neurobiology.

However, as many of my friends and colleagues questioned my sanity in rejecting Boston for the apparent physical and intellectual wilderness of Utah, I did have serious misgivings. These were only enhanced when Harriet Goff, a former UCLA student and housemate who generously agreed to accompany me on the drive there, and I, rather than taking the major north or south highway routes to Utah, chose to strike out across the backcountry of Nevada. There we encountered a huge eagle snacking on its prey in the middle of the road. After spending the night at a hotel in Tonopah, which could have been the set for the TV series *Gunsmoke* and where ancient cowhands rocked on the veranda, I nervously awaited my fate. The next day, after passing signs in barren wilderness that indicated that Salt Lake City was only 16 miles away, you cannot imaging my relief when a real city finally came into view.

The several years in Salt Lake City were ones of wonderful scientific discovery. The ciliary ganglion lived up to the expectations of Guillermo, and our ability to record extracellularly from two discrete but homogeneous populations of neurons embedded in the same ganglion, allowed us to make

rapid progress. Following preganglionic nerve section in adult pigeons, we were able to demonstrate selective reinnervation of these populations by their preganglionic inputs, confirming, with electrophysiology, experiments made many years before by Langley using visual observations of sympathetic reflexes. Turning to development, and using chick embryos, we followed the formation of functional synaptic transmission between vertebrate neurons for the first time and found that it occurred much earlier than one would have anticipated from anatomical and electron microscopic (EM) evidence.

I was also challenged to record from smaller and smaller embryos, honing dissection skills that would later serve me well. More importantly we found that each population appeared to be selectively innervated from the outset, which ran counter to the dogma of the time. Although Sperry had articulated his chemospecificity hypothesis in 1963, it was largely based on regeneration experiments and only the final pattern of retino-tectal innervation had been characterized. It was not at all clear how this developed over time. Did neurons selectively grow to and recognize their targets from the outset by reading molecular cues or did the precise pattern of connections emerge following trial and error establishment of connections and a process of validation? Our results clearly pointed to the former.

We next attempted to address the importance of the target in specifying the properties of the presynaptic neuron, trying to distinguish between Sperry's hypothesis of two prespecified sets of neurons that connected via chemical markers and Paul Weiss' idea that the target imposed its identity on the innervating neurons (myotypic specification). Although the ciliary ganglion was the perfect place to test these hypotheses, my embryonic surgeries to remove the optic vesicle, the target of the ciliary ganglion, prior to innervation, were initially a complete disaster. Although I am often considered to be a developmental biologist, I spent a huge effort and many eggs in trying to duplicate the procedures of experimental embryologists such as Viktor Hambuger with little success. In the end, persistence won out and although these studies were not completed until I had established an independent laboratory at Yale, we were able to show that many features of these two populations developed independent of their target, including their selective innervation by preganglionic afferents.

We carried out these experiments with great enthusiasm, often working late into the night. Although Bob Martin and Cuy Hunt had moved on, Ed Perl and Motoy Kuno were there and our lab and Motoy's spent considerable time together. We shared a special camaraderie in part because as non-Mormons we were essentially outside the mainstream of Salt Lake City society. It was there that I first felt what it must feel like to be an excluded minority. Although everyone was polite and friendly, the non-Mormon, university crowd more or less existed as a small separate town within a larger city. Being a 25-year-old unmarried woman was also viewed

as unusual if not downright unnatural. Fortunately, Guillermo's Australian postdoctoral fellow Peter Vaughan and his wife Sue became my steadfast companions. While dining in my eighth floor apartment, which looked out on the lights of Salt Lake City and the lake beyond, we would pretend we were in some more cosmopolitan place like San Francisco.

Nonetheless I became totally captivated by the exquisite beauty of the mountains. Having spent much of my youth surfing and exploring the wonderful beaches of California, I had developed an almost mystical attachment to the ocean and could not envision living any distance from it. However, the mountains and high deserts of Utah ensnared me with their own unique beauty, making me a lifelong lover of the southwest. We spent many days skiing or hiking, sometimes climbing various peaks with the Wasatch Mountain Club, always beginning early in the morning so that we could scale them and get back down before the inevitable afternoon thunderstorms sent their lightning bolts our way. Toward the end of my stay in Utah my friendship with Guillermo deepened and he became my long-term partner in life as well as in science.

A West Coast Personality Encounters Yale

By 1971 Guillermo had begun to explore positions elsewhere as the physiology department began to disintegrate with Ed Perl and Motoy Kuno leaving for the University of North Carolina. I was contacted by Melvin Cohen to ask if I would be interested in considering a faculty position in the Yale biology department, as they were seeking someone working on neural development. My first inclination, after consulting a map to find the exact location of New Haven, was to say that I was not interested in a position on the east coast. However, Guillermo encouraged me to go for the experience of interviewing and presenting a formal seminar. I arrived in New Haven on a gloomy late spring day as the daffodils were just emerging. I enjoyed giving the seminar and meeting with the scientists there and was much more relaxed than if I had really been seeking this position. I was thus totally surprised and somewhat chagrined, when at the end of my visit I met with the chairman Clement Markert, a gruff but as I was later to discover straightforward and trustworthy person, who offered me the job. He said, however, in a take it or leave it manner, that I had only 2 weeks to accept or decline.

Upon returning to Utah, I vexed over this decision for the full period and only phoned Clem at 4:45 PM on the final Friday to accept the offer. I had decided that beginning my career at a top university such as Yale could not be all that bad and that I could always return to the west coast later when my academic career was under way. Little did I realize then that I would spend the next two decades in Connecticut. This decision also impacted on my personal life as Guillermo was offered a position at the

University of Connecticut, Storrs about the same time. Finally, witnessing today the often long and discouraging struggle that many young scientists endure to find an appropriate position, I realize now how very fortunate I was.

The Yale biology department provided a fine environment for me to grow into an independent scientist, as I describe later. The larger institution of Yale, however, was a strange and anachronistic place for someone who had grown up with the social and intellectual fulmination of California in the 60s. Although the science departments, having recruited faculty from many large state universities, were more geographically diverse and democratic, the rest of Yale seemed both elitist and very staid. Due to the dearth of women there in 1972, I being one of only two in a departmental faculty of more than 30 and female undergraduates having been admitted only a few years earlier, I was asked to serve on numerous university committees. I must confess that some of the views articulated in these committees were quite shocking and could have been the basis of litigation had they become public.

I was also initially amazed that our undergraduate biology students received no academic credit for the large number of laboratory hours they were required to fulfill. It was only much later that I came to appreciate that much of Yale viewed experimental science not as a true intellectual endeavor but rather as something you did with your hands and thus decidedly "working class." A critic was more admired than someone who actually created things, whether this was a new scientific discovery, a painting, or a music composition. Happily, after many years of struggle, our faculty was able to have this course rule modified. Also, unlike many nonscience departments, we never held meetings or dinners at Mory's, where women were only allowed if accompanied by a male. Finally, having puzzled for some time about the strange, windowless buildings scattered about campus that resembled mausoleums, I was at first incredulous when someone told me that these were secret societies. Skull and Bones and several presidents notwithstanding, at that time I could not conceive that such entities could exist in the 1970s.

But, returning to science, it was a thrill to set up one's own laboratory and I quickly attracted two graduate students who differed in many respects; Sheryl Scott came from a southern, upper middle class background and attended Duke University and Deborah Morris was a young black woman, who had grown up in New Orleans and whose father was a preacher. Although there was no overt racism, Yale had to be a challenging and uninviting environment for Debbie, who nevertheless managed admirably by having an ironic sense of humor that allowed her to laugh at and thus deflect various slights and condescending comments. Although they had very different personalities, they were both exceptional students who came to appreciate and respect each other.

Feeling that it was important for a student to take both full credit and responsibility for their initial work, I insisted, idealistically and perhaps naively, that my first students, who later came to include Marcia Honig, Betty Ferguson, and Michael Vogel, have at least one sole-authored publication. Sherry's work turned out to refute the proposal that reinnervation by the original nerve could functionally silence otherwise anatomically normal synapses on goldfish extraocular muscles. I focused on studies on the ciliary ganglion, still in collaboration with Guillermo, in which we defined many aspects of naturally occurring cell death, a phenomenon that was just coming to be appreciated. Debbie had yet to settle on a project, so together we began work on the chick spinal cord, an area that would occupy much of my subsequent scientific effort.

The limb innervating segments of the vertebrate spinal cord had been the subject of numerous studies by the early experimental embryologists, including Viktor Hamburger, Paul Weiss, Sam Detweiler, and George Szekely. They had used surgical manipulations of chick and amphibian embryos to address whether the identities of motor and sensory neurons were prespecified or determined by their targets and whether axons were selectively guided to their targets or whether specific connectivity later arose by a rather poorly defined phenomenon termed "functional validation." The questions and concepts had all been articulated clearly by these pioneers, yet they lacked the experimental tools to unambiguously interpret the many interesting experiments they had carried out. At that time there were no anatomical, tract tracing methods such as the multicolored fluorescent, lipid soluble dyes of today and Jennifer and Matt LaVail had not yet invented retrograde labeling by horseradish peroxidase (HRP). Because multiple spinal nerves converged at the base of the limb in a plexus, there was no way to trace the paths of individual unmyelinated axons among the many thousands growing into the limb.

I quickly realized that this was a problem to which my skills in electrophysiology could be effectively applied. By stimulating individual spinal nerves and by recording from muscles or muscle nerves just as targets were being innervated, Debbie and I were surprised to discover that the many thousands of motor axons grew into the limb with amazing precision, at least at the segmental level. This study published in 1975 ran counter to the accepted ideas of the time, as articulated later in a 1978 paper by Horder, and which viewed axonal guidance as essentially passive. I myself had expected to detect many mistakes with the hope of then discovering the mechanisms by which they were removed. A few years later, after adapting the retrograde HRP labeling method to an in vitro spinal cord-hindlimb preparation, I was able to demonstrate the selectivity of axon outgrowth at the level of the individual motoneuron pool. I have often wondered why the idea of active axon guidance was so hard to find acceptance. I can only surmise that without the awareness of the vast array of guidance and

recognition molecules that we now know exists, the problem of guiding a diverse array of axons over relatively long distances to their divergent targets seemed one of impossible complexity.

Our critics correctly noted that our results were compatible both with active axon guidance, as well as with motor axons maintaining topographic order while being passively guided to their targets. The next step then was to challenge motoneurons by surgically displacing them from their targets. This task was first accomplished by Debbie Morris, who transplanted supernumerary limbs onto chick embryos and thus caused motoneurons to grow into foreign limb regions. I still remember Debbie insisting on driving to Albany in a raging snowstorm so that she could learn from John Saunders, an early pioneer of developmental biology and pattern formation, how to accomplish this. Unfortunately his technique, which produced the well-formed digits he needed to assess anterior-posterior pattern formation, resulted in grossly malformed thighs, and was thus useless for our experiments. Upon her return she switched to Viktor Hamburger's published techniques and eventually succeeded. More informative however, were the surgical deletions and reversals of regions of the neural tube carried out by Cynthia Lance-Jones, when she joined the laboratory as my first postdoctoral fellow. These studies, which were based on a truly heroic number of very difficult embryonic surgeries carried out by Cynthia, were published in four papers in 1980–1981 and have come to be considered as classics.

We were able to demonstrate that motoneurons had their identities specified prior to axon outgrowth, that their axons underwent a dramatic reordering in the plexus region as they sorted into muscle-specific fascicles, and that they could find their targets even when entering the limb from abnormal locations. It seemed to us that these observations could only be explained by differences in the set of cell surface molecules expressed by discrete pools of motoneurons and by motor axons being able to respond actively to guidance cues within the limb. For readers who have experienced frustration with the reviews of their manuscripts, I only note in passing that when the latter two papers of this series were communicated to the Proceedings of the Royal Society by Stephen Kuffler, who had found them excellent and thorough, one of the reviewers found them to be "as a whole marginal and trivial with one portion uninteresting and the other better in concept but poorer in performance." Initially deflated and quite upset, we persisted in more calmly rebutting the criticisms and the papers were eventually published. So my advice would be that if you truly believe you have something important to say, stay the course.

Subsequent studies by another postdoctoral fellow, Kathy Tosney, further explored such active pathfinding and allowed us to visualize the changes in growth cone morphology as axons made specific pathfinding choices. Our studies, together with those of David Bentley and Corey

Goodman who were able to follow the growth cones of individual, identified grasshopper neurons and Christine Holt, who assessed early tectal projections by *Xenopus* retinal cells, gradually changed the mindset, and the concept of active pathfinding is today accepted without question. It has been heartening to see some of these early predictions experimentally validated by Tom Jessell, Sam Pfaff, and their colleagues, who discovered the transcription factor codes that define motoneuron identity and by the laboratories of Corey Goodman, Marc Tessier-Lavigne, and many others who discovered an abundance of guidance cues and their receptors. Yet even today we do not possess a full molecular understanding of how individual motoneuron pools are specified and pathfind to their targets. However, with the wonderful array of genetic and molecular tools now available, this seems likely to be achieved in the near future.

A second line of research, pursued throughout my subsequent career, was also begun at Yale. This sought to understand when and how spinal cord circuits become electrically active during embryonic development and to understand the role, if any, that such activity plays in circuit formation and refinement. Most functional studies of the spinal cords of higher vertebrates had up until that time been carried out in vivo in cats, as isolated cords did not survive well, presumably due to anoxia. It was thus a surprise and a delight to observe one evening as I removed the pins, which had been holding down an isolated spinal cord hindlimb preparation and which had been incubating to allow for retrograde HRP transport, that the cord produced a series of clearly patterned stepping movements of both limbs. Electromyographic (EMG) recordings proved that these were in fact highly patterned, but unfortunately there was no one else around that evening with whom to share my excitement. A short time later, Michael O'Donovan who had been doing cat spinal cord physiology as a postdoctoral fellow with Bob Burke at NIH, joined the lab to try to elucidate this locomotor-like activity of the embryonic chick spinal cord.

We were frustrated initially because only occasionally did cords exhibit such activity and Michael spent several months applying all combinations of possible neuromodulators to elicit movements, before coming to the important realization that adequate oxygenation was the key. His work then revealed that each motoneuron pool exhibited a highly stereotyped bursting pattern and that this appeared autonomous to the pool and was not altered when motoneurons were surgically forced to innervate foreign muscles. Thus, this physiological signature could be used to identify motoneuron pools following experimental manipulations. These observations also clearly refuted the myotypic specification hypothesis proposed earlier by Paul Weiss. Anne Bekoff, working with Paul Stein and Viktor Hamburger at Washington University, had even earlier shown similar patterned activity when recordings were made from muscles in ovo and thus from intact embryos.

Michael went on as an independent scientist to elegantly elucidate the spinal circuits underlying this spontaneous bursting activity, which we now appreciate is widespread throughout the developing nervous system, and due to the work of Carla Shatz and others we know that it plays a role in circuit refinement. In contrast, I for the most part used this bursting activity as a tool to investigate developmental questions. Because molecules that uniquely define each motoneuron pool have to this day not been identified, these physiological signatures are still of enormous help in identifying pools following experimental manipulations. In fact, Gartz Hanson, who has just completed his Ph.D. with me, is currently using such recordings to elucidate changes in motor axon fasciculation and pathfinding that he discovered were produced by altering the frequency of this early spontaneous activity.

Other Activities at Yale

In addition to the research I have briefly described, I immensely enjoyed mentoring graduate students and seeing them emerge as independent scientists and colleagues. Although they are sometimes frustrating, much as one's own teenage children, each is unique in their strengths and mentoring needs, and in the end most seem to find their way. We had a great group of graduate students at Yale and in addition to my own, I was privileged to have served on the dissertation committees of many others who went on to excellent scientific careers; these include John Thomas, Elliot Meyerowitz, Howard Lipshitz, Mark Tanouye, Tom Park, and my current Case colleague Peter Harte. I also had at Yale the most satisfying teaching experience of my career. Naively noting at a faculty meeting how inappropriate it was that we had no systems physiology when most of our undergraduate majors were pre-med, I was immediately asked to design such a course. Because it was not a required course I did not have to cover any fixed set of material and thus selected each year what seemed to be the most interesting physiological problems for which solutions were being found. We read and discussed primary literature and the exams required that the students apply the knowledge that they had acquired to solve problems.

It was a challenging course and not for everyone, but word travels quickly among undergraduates at Yale, and before long the students signing up were always those who valued what I was trying to achieve. I was pleased when, ending a pre-exam, evening review session that was running late, I noted that they did not have to worry about question X as it would not be on the exam. To which someone replied "who cares about the exam? We were up past midnight last night arguing about this question." It was especially gratifying when I encountered a number of these students later, when giving seminars at universities where they were either medical or

graduate students and they told me how important the course had been to them, even though many had not realized it at the time. Although formal mentoring of faculty was nonexistent, I myself benefited from excellent career advice from a number of more senior faculty members including Joe Gall, John Trinkaus, Joel Rosenbaum, and of course one of my most ardent and consistent supporters, Guillermo.

Finally, it was not all work. We had many social activities including a wine and cheese hour every Friday afternoon. The laboratory went on numerous hikes and picnics, some along very scenic parts of the Connecticut River. There were also lovely evenings watching the sunset as boats came into the Noank Harbor, while we dined on fresh lobster and clams at the outside picnic tables at Abbot's. I also enjoyed being a fellow at Branford College during the reign of John Trinkaus as master. Trink was a real rebel who enjoyed shocking the establishment and we were all amazed that the, in our view, staid Yale administration had selected him to be a live-in master. Yale students live from their sophomore to senior years in individual residential colleges. However, Trink and his French wife Madeleine provided scintillating conversation and political discourse at the monthly sherry gatherings at their quarters prior to dining in the college. When a new master replaced Trink upon his retirement, I found the atmosphere at these gatherings so stultifying that I attended only a few with the result that my fellowship was later withdrawn.

It was also at Yale that I first got to know and then become a close friend of Viktor Hamburger, who was then chair of the biology department at Washington University in St. Louis. Viktor, whose life was to span an entire century, had developed the embryological tools that I was able later to so effectively apply to problems in neuroscience. Having come of age in Germany at the end of the First World War, he was to experience the subsequent isolationism imposed on Germany and the eventual rise of the Nazis. He also personally knew Hans Spemann and Hilde Mangold of embryonic organizer fame, having been a student of Spemann and an admirer in both science and life, of Hilde, who died tragically of an accident early in her career. We got together each spring when he visited his daughter Carola, who was then a Classics Professor at Wesleyan University and married to Paul Greengard and who lived within blocks of the biology department. I fondly recall dinners at their home, when Viktor described some of these early experiences, both political and scientific, and thus helped bring history to life.

Later, when hosting a special lectureship for him at the University of Connecticut, I became concerned when he remained very nebulous about his return flight to St. Louis. As it turned out, Viktor, who was then in his mid-80s, had a lady friend flying up from Baltimore and they planned to spend the weekend in Vermont seeing the fall colors. The car rental agent at the airport was quite shocked when a very elderly Viktor ambled

up with his cane to rent the car, and I spent the entire weekend waiting for a call from the Vermont police reporting a car accident. In the end, I got a gracious note from Viktor thanking me for the wonderful weekend they had spent seeing the fall leaves. I last met with Viktor when I was invited to give the annual Hamburger Lecture at Washington University in 1993 when he too was at the age of 93. Having recently had surgery to replace the hip joints that he had replaced many years before, he was unable to attend the seminar but asked to meet with me at his home. I expected to find him very infirm and frail, but quite to the contrary, he entertained me in his living room with appetizers and an aperitif. He then asked "What are you up to scientifically these days?" and as I began to recount some of our recent experiments, I could see him becoming increasingly impatient until he finally blurted out waving his hand "Oh, that's all in that paper you already published. I want to know what you are doing now." That was so typically Viktor and I will always be immensely grateful that I got to know this wonderful scientist and gentleman. We continued to correspond and my last note from Viktor in 2000 thanked me for remembering his 100th birthday. I was also honored to participate in the Society for Neuroscience Symposium held in his honor that year, which was thoughtfully organized by Ron Oppenheim, one of Viktor's early students.

Motherhood and My Decision to Leave Yale

Contemplating motherhood for a developmental biologist has to come with some qualms as one is aware from direct experience of the high incidence of developmental mistakes that arise as we complex organisms translate our genetic code into cellular interactions and morphogenesis. However, it was wonderful news to learn that I was expecting, that the amniocentesis results looked good, and that we would have a son who would likely be born in March. Also this news coming shortly after I was awarded tenure, although it was not planned that way, removed the stress of worrying about having to seek another position. Following the birth of our son Gabriel Roman Pilar on March 9, 1979, I spent a month in the large Victorian home we had purchased in the village of Coventry, not far from the University of Connecticut and notable for being the birthplace of the revolutionary war hero Nathan Hale. This month was considerably more stressful than childbirth itself, as I tried to complete an overdue chapter for the *Annual Review of Neurosciences,* which Max Cowan had assured me could be late under the circumstances, while trying to entertain my mother and learn the essentials of early parenting. With the *Annual Review* production editor badgering me it seemed every other day, I finally completed the chapter and returned to the relative peace of work at Yale. I continued to maintain an

apartment in downtown New Haven where Gabe and I spent the week, returning to Coventry to enjoy weekends hiking, swimming, or kayaking in nearby lakes and also where Guillermo and I undertook a decade-long renovation of our 1860 era home.

Organized day care was nonexistent at that time in New Haven, but I was incredibly lucky to have Gabe cared for by the family of Jahja Ling, now the music director of the San Diego Symphony, who was at that time studying for a Fine Arts degree at Yale. First his sister Martha and later his mother, who was like a real grandmother, provided Gabe with care that left me free from worries. He also became fluent in Mandarin. I know all working mothers have doubts about what they could have done better, but I have come to be fairly convinced that most of a child's personality is defined by genes and that we can tweak this only slightly. In fact Jahja's mother, realizing that good motherhood could only do so much, encouraged me to have three children despite being very busy. That way, she would say, you have a good chance that at least one will turn out well.

Our son has grown into a fine young man and he would have to say if he missed out on anything. He did spend a lot of late afternoon hours in the lab, discovering a myriad of uses for dry ice, including making bombs by enclosing it in rubber pipette bulbs and using it as a propellant for shooting Eppendorf tubes from plastic syringes. He also got a useful early and sophisticated introduction to computers from a variety of students, postdoctoral fellows, and even faculty members. However, at one point as I was rationalizing a trip to give a seminar by saying how one had to live up to the commitments that one has made, he dryly commented "Mom, there are lots and lots of neuroscientists. I'm sure they can find someone to replace you." So much for feeling self-important! However, compensating partly for the inevitable parental absences that accompany a scientific career has to be the exposure that our children have to students and colleagues from countries and diverse cultures all over the world. Accompanying me at the age of 8 on a 3-week course I gave in Mexico City in 1987, he was totally impressed with the pyramids and with the long and rich history of Mexican art and culture, sagely noting how relatively impoverished the United States seemed in comparison.

Nevertheless, after almost 3 years of shuttling back and forth each week between Coventry and New Haven, I realized that Guillermo and I had to find positions in the same place. Also downtown New Haven was not the place to raise a child. As an adult I had adapted to its high crime rate, but I could not consider bringing up a child in an environment where all strangers had to be viewed as potential threats. Although several possibilities for relocation emerged, in the end I left Yale in 1983 to take a faculty position in the Section of Regulatory Biology that later became the Department of Physiology and Neurobiology at the University of Connecticut, Storrs, and which Guillermo chaired.

The University of Connecticut and My Introduction to Molecules

Many were surprised by my decision to leave Yale, especially after being promoted to Professor, for the relative isolation of Storrs, which was set among cow pastures in rural Connecticut. However, several colleagues from Harvard, who subsequently left that institution, later confided that this move had reassured them that if there was life for me after Yale, so too could there be life for them after Harvard. Our personal life, however, was far saner and although I missed many friends and colleagues at Yale, the neuroscientists with whom I interacted daily provided a stimulating environment. It was during my stay at Storrs that I also overcame my fear of gels and biochemistry. This was due largely to Jon Covault, a newly hired faculty member from Josh Sanes' laboratory who was practicing state of the art biochemistry applied to the nervous system. Prior to molecular biology and the development of monoclonal antibodies, the heterogeneity of the nervous system had made biochemistry extremely difficult and slow going. I had long wanted to find a molecular basis for the selective axon fasciculation and pathfinding events we had been studying, but at that time the only known cell surface molecules that seemed potential candidates were NCAM and L1 (then called NILE).

A truly catalytic event in helping me to bridge the divide between cellular interactions and molecules was the 1983 FASEB meeting in Saxton's River, Vermont. Urs Rutishauser, who had recently left Gerald Edelman's group at Rockefeller for a position at Case Western Reserve University in Cleveland, spoke of his recent work on both NCAM and on a large carbohydrate modification of NCAM, called polysialic acid or PSA, which appeared to modulate the adhesive properties of NCAM and axon fasciculation at least in culture. I spoke on axon fasciculation and sorting in the plexus region of the in vivo chick embryo, and we both saw the potential importance of bringing these two lines of research together. Later we talked with great excitement, as we sat under a waterfall in a stream that ran through town, of all sorts of potential experiments in which either NCAM function or its level of sialylation could be modified in vivo during embryonic chick development. We were brought back to earth as we emerged from the stream by the discovery that we were both covered with leeches. Thus began a long and fruitful collaboration.

The actual experiments had to be postponed for several years to await the perfection of reagents that would allow us to modify NCAM function or PSA expression in vivo. These included high-quality anti-NCAM Fab fragments from Urs' laboratory as well as an endosialidase that could specifically degrade the sialic acid residues on PSA in vivo. This exceptional tool was made by Eric Vimr and his colleagues, while studying the PSA that coats some classes of bacteria, including the meningococcal strains

that cause meningitis. Eric fortunately brought this tool to the attention of Urs, and thus more broadly to the neuroscience community, by forcefully insisting on a Thanksgiving Day meeting at Urs' home while he was in Cleveland visiting relatives. Fortunately for our collaboration, Urs reluctantly relented. Equally important were antibodies that would enable us to visualize what our experimental perturbations were doing to PSA expression in the developing embryo. Unfortunately, PSA is very nonantigenic, but eventually both Tom Jessell and Jane Dodd at Harvard and Genevieve Rougon's group in France succeeded in preparing monoclonal antibodies that recognized this carbohydrate. Finally, the experiments that Urs and I planned in the stream at Saxton's River could proceed.

It is hard to convey how exciting it was at that time to be able to modify the function of NCAM or the expression of PSA in a defined way in space and time in the intact embryo by very local injections of antibodies or endosialidase and discern clear and reproducible alterations in axon fasciculation and pathfinding. This also marked my first use of immunostaining and I remember how Urs and I had to drive with the first batch of slides we had stained to the Biobehavioral Sciences Department where Enrico Mugnaini had the only fluorescent microscope around. Times have certainly changed! Although the power of *Drosophila* genetics was already apparent, genetic approaches to achieve in vertebrates what we desired were at that time not a viable option.

It became quickly apparent that the characteristic pattern of intramuscular nerve branching in slow and fast muscle regions could be differentially modified by altering the function of NCAM or L1/NgCAM, or by removing PSA. These observations would not have been possible without a whole-mount muscle preparation in which the entire detailed nerve branching pattern was beautifully revealed. Lisa Dahm, my first graduate student at UCONN, developed this preparation as she explored activity-dependent alterations in intramuscular nerve branching. She proposed that these were responsible for the rescue of motoneurons during the programmed cell death period, which Randy Pittman and Ron Oppenheim had previously shown followed blockade of neuromuscular activity. The monoclonal antibody, which was essential for the success of this preparation, had been made by Hideaki Tanaka, who joined me as a postdoctoral fellow just as I was leaving Yale. Rather than being disappointed, both he and his wife were immensely relieved not to be moving to New Haven, where the high crime rate that they had read about, especially in comparison to the safety of Japanese society, had caused them great concern. Even using this antibody, Lisa found that frozen cross sections could only reveal an apparent increase in axons and a more disorganized pattern of growth. It was only through visualization of the entire branching pattern that we could make specific detailed hypotheses that could later be confirmed with quantitative measurements of branching.

Polysialic acid, the expression of which is dynamically regulated during development and is also sensitive to the pattern of electrical activity, was subsequently shown to play key roles in multiple developmental events. We provided strong suggestive evidence that it was responsible for the different patterns of axon growth in fast and slow muscle regions. This idea was later confirmed at Case by my postdoctoral fellow, Vic Rafuse, when he ingeniously combined the removal of PSA with the creation of chick-quail chimeras to cause "fast" quail motoneurons to innervate slow chick muscle. This experiment in turn built on Hideaki Tanaka's previous study showing that muscles in these chimeras were selectively innervated by the homologous foreign motoneuron pool. The work of another graduate student, Jicheng Tang, subsequently showed that PSA was also responsible for the dramatic defasciculation of motor axons in the plexus that Cynthia and I had described many years earlier, and that its removal resulted in motor axon pathfinding errors. Recently and more than a decade later, Gartz Hanson has identified PSA as a prime molecular candidate to explain the pathfinding errors that he discovered were caused by altering the frequency of early spontaneous activity.

Although I think we all try to carry out our experiments with great care and interpret our results conservatively, it is always reassuring to revisit a problem many years later, often with newly available tools, and to be able to both confirm the original findings and extend new concepts. It is often those older observations that suggest further decisive experiments. Thus, I am chagrined by the tendency of many young scientists today to consider anything not published in the last few years to be obsolete and who only read earlier work in abstract form if at all. I think that they are missing many biological gems that could be so effectively mined by the tools of today.

Another enjoyable activity, begun at Yale but carried out throughout my time in Storrs, was to participate in the Cold Spring Harbor summer course in developmental neurobiology that was organized by Dale Purves (later replaced by Corey Goodman) and Paul Patterson. The ferry ride from Bridgeport, Connecticut to Port Jefferson, New York was always refreshing and I loved being able to interact with a truly fantastic group of students, which included Marc Tessier-Lavigne, Susumu Tongawa then of recent Nobel Prize fame as he switched his career to neuroscience, and many others. There were of course also always a few laggards. It is hard to imagine two individuals so different in their approach to science or life as Dale and Paul, yet this dichotomy made for especially lively discussions and the exploration of diverse explanations for given findings.

I also remember some "wild" parties and wading in the Sound at night at the Banbury Campus where the course took place. We were clearly a bit too rowdy for the very formidable manager of the mansion there, a Mrs. Greene, if I remember correctly. I do remember my extreme

embarrassment when, despite my protests, she insisted in carrying my luggage into the mansion where I stayed one time. One of the downsides to living in two places (my New Haven–Storrs days) is that needed items are often at the other location. Thus, finding no suitcase at my New Haven apartment, I had packed my belongings in a brown paper shopping bag, which was ceremoniously carried by her into the mansion.

In 1989, I was very fortunate to be invited to be a visiting Weirsma Professor at Cal Tech, while Guillermo was able to carry out a sabbatical with Henry Lester there. This provided the possibility to interact with a strong and diverse group of neuroscientists that included David Anderson, Paul Patterson, Mark Konishi, Mary Kennedy, and many others. I set out to utilize the state of the art monoclonal facility run there by Susan Oh, to use new immune suppression techniques to try to raise antibodies that would selectively recognize subsets of lumbar spinal motoneurons. Although not successful in this, I did raise a number of antibodies that were very useful to me in later research. I worked in Paul Patterson's laboratory, which had a fine group of more molecularly oriented students and postdoctoral fellows that included Zaven Kaprielian, James Sabry, and Tetsuo Yamamori. Interacting with them and doing biochemical fractionations, gels, and dot blots with my own hands further helped in demystifying molecular biology, as did getting to sit in on David Anderson's lab meetings.

This period also provided a wonderful opportunity for our son to become better acquainted with his grandparents, who lived in different locations in California, having separated about the time I entered high school. Many weekends were spent at the beach in Carlsbad where my mother lived and where I taught Gabe to boogie board and body surf, or at the desert near Joshua Tree National Monument, where we hiked, shot off rockets, or practiced target shooting with my dad. Although I loved the diverse activities possible in California just as I had as a child, the traffic, which made getting to these locations a nightmare, and the extreme overdevelopment that seemed to have ruined the California of my youth caused me to stop considering that I might one day return there. Learning that the modest stucco house in Pasadena that Cal Tech had graciously made available to us free of charge was valued, even at that time, at $450,000 cemented this decision. It was, however, a year of intellectual renewal that I will always treasure.

Returning to Connecticut, I began to become dissatisfied by the vision, or rather lack thereof, of the higher administrators at the University of Connecticut at Storrs. Seeing the potential of mouse genetics for future neuroscience research, I was dumfounded when the dean at that time, also a biological scientist, informed me that land grant universities such as ours could not expend the resources needed for mouse genetics and that we should content ourselves with using simpler organisms such as the slime mould *Dictyostelium*. Although I appreciated this organism as a fine model

for many developmental and cell biological processes, its lack of a nervous system was clearly problematic for a neuroscientist.

Thus, although not actively seeking to relocate, I was ready to consider the possibility when in 1991 my colleagues invited me to give a seminar at Case Western Reserve University in Cleveland. Case had a very strong group interested in adhesion/guidance molecules and axon growth, which included Vance Lemmon, Jerry Silver, and my long-time collaborator Urs. Further, much to my surprise, I found Cleveland to be an attractive and very livable city. Thus, when Story Landis, the first chair of the recently created Neurosciences Department in the School of Medicine, indicated during my visit that a senior faculty position might be available in the next several years, a seed was sown that later changed my life in many ways. Both Story and Urs worked to come up with an attractive offer and in the summer of 1993 our son Gabe and I moved to Cleveland. Due to grant-related issues, Guillermo was unable to join us for several years.

Cleveland and My Introduction to Bioinformatics and Administration

Before leaving Connecticut, I was fortunate to be joined by two very fine postdoctoral fellows, Victor Rafuse from Canada and Esther Stoeckli from Switzerland, who greatly facilitated the move and ensured that experiments were up and running shortly after our arrival in Cleveland. As neurosciences space was still being consolidated, Hunt Willard, then Chair of Genetics, generously offered me space in the newly constructed Biomedical Research Building where Urs, who had become a member of the Genetics Department, was located. This edifice, like so many new research buildings, was designed in an open space mode, which efficiently lets administrators, who have no real understanding of the space needs of different types of science, titrate space to match grant income, as well as to more densely pack in researchers. Although my lab and Urs' had a combined critical mass adequate for scientific interactions, the space was poorly designed for either fluorescent microscopy, which was then our major technique, or electrophysiology. This necessitated that we use the few separate rooms for microscopy and carry out relatively delicate electrophysiological recordings in the, albeit wide, hallway with people slamming the adjacent cold room door or pushing by us to get down the corridor. Thus, we were all delighted to move several years later into windowless and somewhat more antiquated space in the Neurosciences Department, where we currently still reside.

From a personal perspective, our son was not eager to move to Cleveland. Having grown up in New England, his two major loves were sailing, and clearly Lake Erie did not measure up to the Atlantic, and skiing, where northeast Ohio was also found lacking. However, we purchased a house in Shaker Heights within a 10-minute drive from Case and came to

appreciate the unique social culture of this region, which combines a liberal attitude more like the northeast with a genuine midwestern friendliness. I also have come to value living in a racially integrated town that has the community spirit to maintain an excellent public school system, from which our son greatly benefited. Finally, it was also deemed especially appropriate for me to emigrate from Connecticut to Cleveland, as the northern part of Ohio from the Pennsylvania border to Cleveland was originally the Western Reserve of Connecticut, from whence derives the rather strange name of my current institution.

Scientifically, the two major research themes begun earlier have continued to evolve and extend in interesting ways. First I have never been able to get away from NCAM. Following studies by Barbara Fredette, my last postdoctoral fellow at UCONN, and Vic Rafuse, which revealed that the expression of the different isoforms of NCAM was highly dynamically regulated and activity dependent during chick neuromuscular development, it did not seem that we could make much further progress in the chick system with the tools then available. However, the finding that neuromuscular development appeared to be totally normal in the NCAM null mouse generated by Harold Cremer in 1994 prompted us to obtain some of these mice to see if we might detect deficits that others had missed. I confess that I was partly motivated by people commenting that it was a pity that the very lovely observations we had made in the chick were not relevant to mammalian development. In the end, it took a team effort by Vic Rafuse, Christian Bose, and the very talented electrophysiologist Luis Polo-Parada, and the application of multiple techniques including physiology and the visualization of synaptic vesicle cycling via styryl dyes, to detect novel defects in synaptic transmission and the mobilization of synaptic vesicles. We concluded that NCAM, and specifically its 180-kDa isoform, is required for adult synapses to maintain transmission with repetitive stimulation and that, rather than acting as an adhesion molecule, this isoform with an intracellular domain appeared to be acting as a signaling or scaffolding molecule.

It was again a stroke of luck that brought a Swiss student Florian Plattner, who had just completed a master's degree in biotechnology and bioinformatics in Europe, to my lab to carry out 9 months of hands-on research. Florian helped me to appreciate, and thus begin to use, the vast wealth of data now available on the Internet. Through him, we discovered a highly conserved intracellular domain on NCAM that mediates these effects on transmission and that acts via myosin light chain kinase and myosin driven movements of synaptic vesicles. Thus, I am investigating a whole new area and one where I would not have expected to find myself 5 years ago. This is why science is both rejuvenating and so much fun.

Our studies of activity-dependent phenomena also took a new turn when a M.D./Ph.D. student Louise Milner, who had been investigating the

selective fasciculation of slow and fast motor axons in spinal nerves, decided to determine if this phenomenon was activity dependent. Without my knowledge, she pharmacologically "blocked" spontaneous rhythmic activity during early stages of axon outgrowth and excitedly showed me the data suggesting that this had caused apparent motor axon pathfindng errors. This prompted us to characterize the cord circuits that drove spontaneous activity at these early times as it was not even clear that such cords were rhythmically active. Gartz Hanson then extended the characterization of these circuits to mice and showed that briefly reducing the frequency of such activity produced alterations in motor axon fasciculation, pathfinding errors, and changes in the expression of specific guidance/adhesion molecules. These findings challenge the traditional view that patterned electrical activity is only used later in development to refine circuits assembled via molecular signals. Our plan is to now use multi-photon calcium imaging of single cells to characterize the activity of cord circuits at the earliest stages, when motoneurons are still migrating and extending their axons, and to determine how such activity intersects with already elucidated molecular signaling pathways to lead to the assembly of functional cord circuits. I feel so grateful to be able to be exploring these new areas at my age.

With age and experience, however, comes the responsibility to try to use that experience to help both society and our scientific discipline. I thus have enjoyed serving on a number of advisory panels, both of government and scientific societies. However, I had always avoided purely administrative positions, and it was thus with some reluctance that I agreed to be interim chair of the Neurosciences Department when Story Landis left in 1995 to head the intramural program at NINDS. Story reassured me, who had spent most of my career in biology departments, that our departmental administrator Narlene Brown more or less ran the department, which luckily for me turned out to be true, and that other medical school chairs would provide me with needed advice. It was the highly congenial environment at Case and seeing the fine group of basic science chairs, who included Hunt Willard, Toni Scarpa, Fritz Rottman, and Mike Lamm, work effectively together for common goals that made me feel that I could cope with being a chair. The realization that my ideas, which I often found better than various administrators, could be put into practice also helped.

In 1999, I became actual Chair and for the most part have enjoyed building on the strong department that Story had assembled and extending it into new areas. We have recruited an excellent group of young faculty members and have managed to retain a cadre of senior scientists who are respected leaders in their fields. I was just beginning to become complacent, when our institution underwent a crisis of major proportions; this involved a caustic conflict between the medical school and our major affiliated hospital as well as the turnover of many administrators including the university president, the dean of the medical school, and the head of

the hospital. I was asked to serve on reconciliation committees to try to fix the problems and to search for a new dean, which resulted in several years of emotional stress and having to focus my time and creative energy away from my true interests. However, as we now appear back on track, I find that I can turn my focus back to the department and lab.

New Mexico

This account would be incomplete without an acknowledgment of the importance that New Mexico and the home that we built there in the mountains several hours north of Santa Fe has had for me. Having been captivated by the beauty of the southwest during our sojourns in Utah, Guillermo and I, and later our son Gabe, vacationed there every summer, kayaking several times down the Green River in Utah. However, we came to prefer the ethnic and cultural diversity of New Mexico with its historic Hispanic villages and diverse Native American tribes. Toward the end of camping vacations we would look for land to purchase but could never find any we both liked and could afford. But once, returning from Lake Powell, we were captivated by the mountains and high meadows just south of the Colorado border near the towns of Chama and Tierra Amarilla and purchased a large tract of mixed ponderosa and juniper-pinion forest with amazing views especially from a ridge where the mountains around Abiqui, 40 miles to the south, are clearly visible. We were subsequently quite shocked later that year to learn that in 1967 Tierra Amarilla had been the site of a protest for land rights by local Hispanics, which resulted in the occupation of the county courthouse and the calling out of the state National Guard. However, over the years we have gotten to know and appreciate many of the locals and have had more adventures than I have space to recount. During this time, our son was able to spend wonderful periods free from television and other distractions while he explored nature at its finest. We also established a long-lasting relationship with a Navajo family whose children are like my own grandchildren. It is a place of great tranquility where I can escape each year from the hectic pace of academic life to recharge myself both mentally and spiritually. I envision that one day, when I have had enough, if not of science, then of the chores that accompany its practice today, that I will retreat there to pursue interests such as photography and writing for which I have never found enough time.

Concluding Remarks

I would like to thank the Society of Neuroscience for honoring me with the invitation to contribute this chapter and Larry Squire, who saw the importance of recording the history of our relatively young discipline in its practitioners' own words. I will also take a moment to apologize to the many people who made contributions or who have influenced my career

and whom I did not have space to mention, as well as for any factual inaccuracies that my aging brain has introduced into this account. Having entered science when women were very scarce, I can honestly say that I have never felt out of place, thanks to the support and friendship of not only my mentors but of most of my peers, the vast majority of whom were male, during my days as a graduate student, postdoctoral fellow, faculty member, and finally chair.

Reflecting on both the past and future, the great excitement I feel in seeing neuroscience on the verge of so many important discoveries, is tempered by my concern over the recent rise of religious extremism, political intolerance, and anti-intellectualism in my country. Science too has changed, having lost some of its innocence as it becomes practiced for profit. Furthermore, the hectic pace of activities we seem to need to carry out to survive in science today leaves so little time for scholarly reflection. Finally, the rise of directed research in which large groups are assembled to address specific problems, although having a place, is likely to come at the expense of basic science and individual creativity, which to me will always be the engine that drives biological science. However, these are challenges for the next generation of neuroscientists to confront and hopefully solve.

Selected Bibliography

Bekoff A, Stein PSG, Hamburger V. Co-ordinated motor output in the hindlimb of the 7 day old chick embryo. *PNAS* 1975;72:1245–1248.

Cajal S. *Histologie du Systeme Nerveux de L' Homme et des Vertebres,* vol. 1 and 2. Translated from Spanish by L. Azoulay. Madrid: Instituto Ramon y Cajal, 1952.

Dahm L, Landmesser L. The regulation of intramuscular nerve branching during normal development and following activity blockade. *Dev Biol* 1988;130:621–644.

Dahm L, Landmesser L. The regulation of synaptogenesis during normal development and following activity blockade. *J Neurosci* 1988;1:238–255.

De Castro F. Aspects anatomiques de la transmission synaptique ganglionaire chez le mamiferes. *Archiv Int. de Physiologie* 1951;59:479–513.

Ferguson BA. The effect of dorso-ventral limb rotations on motor projection patterns. *J Neurosci* 1983;3:1760–1772.

Fredette BJ, Landmesser L. Relationship of primary and secondary myogenesis to fiber type development in embryonic chick muscle. *Dev Biol* 1991;143:1–18.

Fredette B, Landmesser L. A re-evaluation of the role of innervation in primary and secondary myogenesis in developing chick muscle. *Dev Biol* 1991;143:19–35.

Fredette B, Rutishauser U, Landmesser L. Regulation and activity dependence of N-Cadherin, NCAM isoforms, and polysialic acid on chick myotubes during development. *J Cell Biol* 1993;123:1867–1888.

Hanson MG, Landmesser LT. Characterization of the circuits that generate spontaneous episodes of activity in the early embryonic mouse spinal cord. *J Neurosci* 2003;23:587–600.

Hanson MG, Landmesser LT. Normal patterns of spontaneous activity are required for correct motor axon guidance and the expression of specific guidance molecules. *Neuron* 2004;43:687–701.

Honig MG. The development of sensory projection patterns in the embryonic chick hind limb. *J Physiol* 1982;330:175–202.

Honig M, Lance-Jones C, Landmesser L. The development of sensory projection patterns in embryonic chick hindlimb under experimental conditions. *Dev Biol* 1982;118:532–548.

Horder TJ. Functional adaptability and morphogenetic opportunism, the only rules for limb development? *Zoon* 1976:181–192.

Lance-Jones C, Landmesser L. Motoneuron projection patterns in embryonic chick limbs following partial deletions of the spinal cord. *J Physiol* 1980; 302:559–580.

Lance-Jones C, Landmesser L. Motoneuron projection patterns in the chick hindlimb following early partial reversals of the spinal cord. *J Physiol* 1980;302:581–602.

Lance-Jones C, Landmesser L. Pathway selection by chick lumbosacral motoneurons during normal development. *Proc R Soc London B* 1981;214:119–152.

Lance-Jones C, Landmesser L. Pathway selection by embryonic chick motoneurons in an experimentally altered environment. *Proc R Soc London B* 1981;214:119–152.

Landmesser L. Contractile and electrical responses of vagus-innervated frog sartorius muscles. *J Physiol* 1971;213:707–725.

Landmesser L. Pharmacological properties, cholinesterase activity and anatomy of nerve-muscle junctions in vagus-innervated frog sartorius. *J Physiol* 1971;220:243–256.

Landmesser L. The distribution of motoneurons supplying chick hindlimb muscles. *J Physiol* 1978;284:371–389.

Landmesser L. The development of motor projection patterns in the chick hindlimb. *J Physiol* 1978;284:391–414.

Landmesser L. The acquisition of motoneuron subtype identity and motor circuit formation. *Int J Dev Neurosci* 2001;19:175–182.

Landmesser L, Dahm L, Schultz K, Rutishauser U. Distinct roles for adhesion molecules during innervation of embryonic chick muscle. *Dev Biol* 1988; 130:645–670.

Landmesser L, Dahm L, Tang J, Rutishauser U. Polysialic acid as a regulator of intramuscular nerve branching during embryonic development. *Neuron* 1990;4:655–667.

Landmesser L, Honig M. Altered sensory projections in the chick hindlimb following early removal of motoneurons. *Dev Biol* 1986;118:511–531.

Landmesser L, Morris DG. The development of functional innervation in the hindlimb of the chick embryo. *J Physiol* 1975;249:301–326.

Landmesser L, O'Donovan M. Activation patterns of embryonic chick hindlimb muscles recorded in ovo and in an isolated spinal cord preparation. *J Physiol* 1984;347:189–204.

Landmesser L, O'Donovan M. The activation patterns of embryonic chick motoneurons projecting to inappropriate muscles. *J Physiol* 1984;247:205–224.

Landmesser L, Pilar G. Selective reinnervation of two cell populations in the adult ciliary pigeon ganglion. *J Physiol* 1970;211:203–216.

Landmesser L, Pilar G. The onset and development of transmission in the chick ciliary ganglion. *J Physiol* 1972;222:691–713.

Landmesser L, Pilar G. Synapse formation during embryogenesis on ganglion cells lacking a periphery. *J Physiol* 1974;241:715–736.

Landmesser L, Pilar G. Synapse formation and cell death during normal ganglionic development. *J Physiol* 1974;24:739–749.

Landmesser L, Pilar G. Fate of ganglionic synapse and ganglion cell axons during normal and induced cell death. *J Cell Biol* 1976;68:357–3784 .

Landmesser L, Szente M. Activation patterns of lumbosacral motoneurons following blockage of functional activity and motoneuron cell death. *J. Physiol* 1986; 380:157–174.

Langley JN. On the re-generation of pre-ganglionic and post-ganglionic visceral nerve fibres. *J Physiol* 1897;22:215–230.

Milner L, Landmesser L. Cholinergic and GABAergic inputs drive patterned spontaneous motoneuron activity prior to target contact. *J Neurosci* 1999;19:3007–3022.

Milner L, Rafuse V, Landmesser L. Selective fasciculation and divergent pathfinding decisions of motoneurons projecting to fast and slow muscle regions. *J Neurosci* 1998;18:3297–3313.

Morris DG. Development of functional motor innervation in supernumerary hindlimbs of the chick embryo. *J Neurophysiol* 1978;41:1450–1465.

O'Donovan M, Landmesser L. The development of alternating activity in antagonistic chick motoneuron pools. *J Neurosci* 1987;10:3256–3265.

Pilar G, Landmesser L. Axotomy mimicked by localized colchicine application. *Science* 1972;117:1116–1118.

Pilar G, Landmesser L. Ultrastructural differences during embryonic cell death in normal and peripherally deprived ciliary ganglia. *J Cell Biol* 1976;68:339–356.

Pilar G, Landmesser L, Burstein LG. Competition for survival among developing ciliary ganglion cells. *J Neurophysiol* 1980;43:233–254.

Polo-Parada L, Bose CM, Landmesser L. Alterations in synaptic vesicle dynamics and the organization of transmitter release machinery in NCAM deficient neuromuscular junctions. *Neuron* 2001;32:815–828.

Polo-Parada L, Bose CM, Plattner F, and Landmesser L. Distinct roles of different neural cell adhesion molecule (NCAM) isoforms in synaptic maturation revealed by analysis of NCAM 180kD isoform deficient mice. *J Neurosci* 2004;24:1852–1864.

Polo-Parada L, Plattner F, Bose CM, Landmesser LT. NCAM acting via a conserved C-terminal domain and requiring MLCK activity is essential for effective transmission with repetitive stimulation. *Neuron* 2005;46:917–931.

Rafuse V, Landmesser L. Contractile activity regulates isoform expression and polysialylation of NCAM in cultured myotubes: involvement of Ca^{++} and protein kinase C. *J Cell Biol* 1996;132:969–983.

Rafuse V, Landmesser L. The pattern of intramuscular nerve branching is determined by the innervating motoneuron and its level of polysialic acid. *J Neurosci* 2000;20:1056–1065.

Rafuse V, Milner L, Landmesser L. Selective innervation of fast and slow muscle regions during early chick neuromuscular development. *J Neurosci* 1996; 16:6864–6877.

Rafuse V, Polo-Parada L, Landmesser L. Structural and functional alterations of neuromuscular junctions in NCAM-deficient mice. *J Neurosci* 2000;20: 6529–6539.

Scott SA. Maintained function of foreign and appropriate junctions on reinnervated goldfish extraocular muscles. *J Physiol* 1975;268:87–109.

Stoeckli E, Landmesser L. Axonin-1, NrCAM and NgCAM play different roles in the in-vivo guidance of chick commissural neurons. *Neuron* 1995;14:1165–1179.

Stoeckli E, Sonderegger P, Pollerberg GE, Landmesser L. Interference with axonin-1 and NrCAM interactions unmasks a floor plate activity inhibitory for commissural axons. *Neuron* 1997;18:209–221.

Tanaka H, Landmesser L. Interspecies selective motoneuron projection patterns in chick-quail chimeras. *J Neurosci* 1986;6:2880–2888.

Tanaka H, Landmesser L. Cell death of lumbosacral motoneurons in chick, quail and chick-quail chimera embryos: A test of the quantitative matching hypothesis of neuronal cell death. *J Neurosci* 1986;6:2889–2899.

Tang J, Landmesser L, Rutishauser U. Polysialic acid influences specific pathfinding by avian motoneurons. *Neuron* 1992;8:1031–1044.

Tang J, Landmesser L. Reduction of intramuscular nerve branching and synaptogenesis is correlated with decreased motoneuron survival. *J Neurosci* 1993; 13:3095–3103.

Tang J, Rutishauser U, Landmesser L. Polysialic acid regulates growth cone behavior during the sorting out of motor axons in the plexus region. *Neuron* 1994;13:405–414.

Tosney K, Landmesser L. Pattern and specificity of axonal outgrowth following varying degrees of chick limb bud ablation. *J Neurosci* 1984;4:2518–2527.

Tosney K, Landmesser L. Development of the major pathways for neurite outgrowth in the chick hindlimb. *Dev Biol* 1985;109:193–214.

Tosney K, Landmesser L. Specificity of motoneuron growth cone outgrowth in the chick hindlimb. *J Neurosci* 1985;5:2336–2344.

Tosney K, Landmesser L. Growth cone morphology and trajectory in the lumbosacral region of the chick embryo. *J Neurosci* 1985;5:2345–2358.

Vogel MW. Activation patterns of embryonic chick lumbosacral motoneurones following large spinal cord reversals. *J Physiol* 1987;389:491–512.

Vogel MW, Landmesser L. Distribution of myosin ATPase fiber types in embryonic chick limb muscles innervated by foreign motoneurons. *Dev Biol* 1986; 119:481–495.