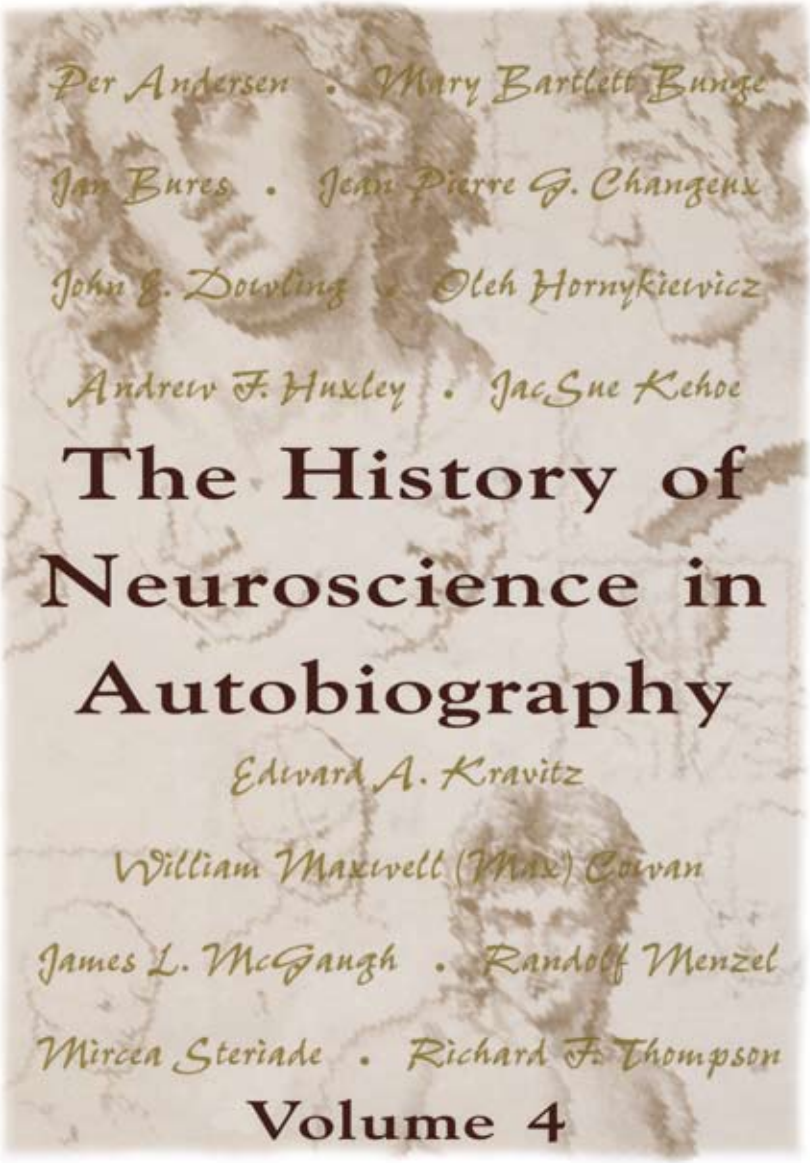



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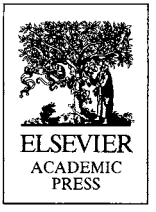
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# The History of Neuroscience in Autobiography

## VOLUME 4

Edited by Larry R. Squire




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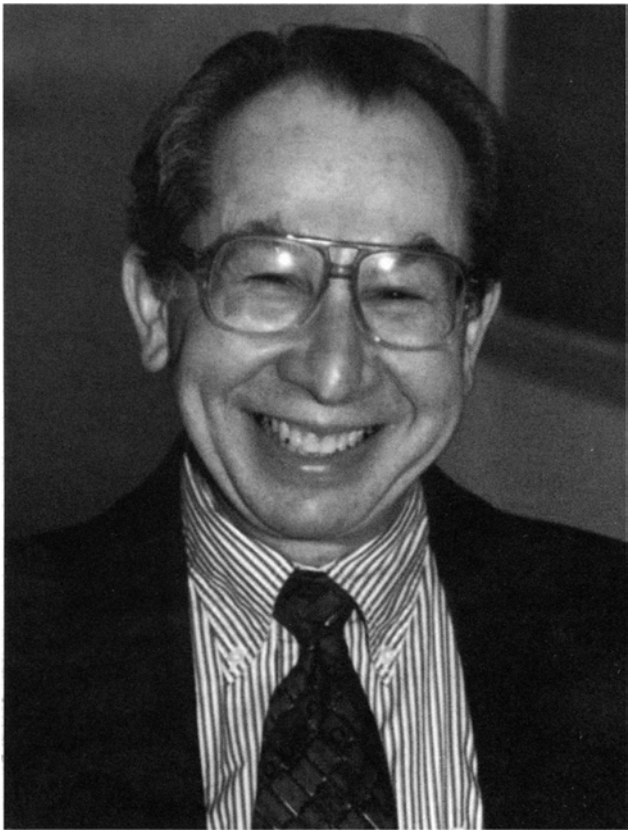
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# *Edward A. Kravitz*

**BORN:**

New York, New York
December 19, 1932

**EDUCATION:**

City College of New York, B.S. (1954)
University of Michigan, Ph.D. (Biological Chemistry, 1959)

**APPOINTMENTS:**

Postdoctoral Fellow, National Heart Institute (1958)
Harvard Medical School (1961)
George Packer Berry Professor of Neurobiology, Harvard Medical School (1986)

**HONORS AND AWARDS (SELECTED):**

American Academy of Arts and Sciences (1976)
Einstein Visiting Fellow, Hebrew University (1981)
National Academy of Sciences, USA (1984)
Institute of Medicine (1986)
Governing Council, Institute of Medicine (1990–1994)
Humboldt Research Award (1992)
John S. Guggenheim Fellowship (1992)
A. Clifford Barger Lifetime Achievement in Mentoring Award, Harvard Medical School (1998)
Education Award, Association of Neuroscience Departments and Programs (2001)

*In his early studies, Ed Kravitz and his collaborators demonstrated a transmitter role for GABA and established Procion Yellow as the first widely used dye for the determination of neuronal geometry. His studies with the amines serotonin and octopamine demonstrated their roles as synaptic modulators and led to studies exploring the function of amine neurons in complex patterns of behavior such as aggression. He has used invertebrate models, first lobsters and recently fruit flies, in order to bring genetic methods to the study of aggression. He has long been committed to education at the clinical/basic science interface and to the education of minorities in the sciences and medicine.*

# Edward A. Kravitz

**My Life up to Now**
"(3rd verse) I get up each morning and dust off my wits
Open the paper and read the obits
If I'm not there I know I'm not dead
So I eat a good breakfast and go back to bed

(chorus) How do I know my youth is all spent
My get up and go has got up and went
But in spite of it all I'm able to grin
And think of the places my get up has been."

"Get Up and Go," Song by Pete Seeger (1960)

If I ever really get old, I will have this song as my anthem. "Get Up and Go" is a wonderful upbeat song about getting older that I first heard in a movie version ("Wasn't that a Time," American Roots Music, producer, 1982) of what turned out to be the Weaver's last performance at Carnegie Hall in 1981. I wish I had been at the concert. I must admit that I was apprehensive when asked to write an autobiography for *The History of Neuroscience in Autobiography, Volume 4*, because unless one is a serial killer or has sex with important people in prominent places, most scientists that I know tend to write their autobiographies near or at the ends of their active scientific lives. I don't feel any place near the end of my active scientific life, despite the attempts of deans and others to hasten the happening of that sorry event. I also worry about how I make anything I write into an accurate record of my career and not an interpretation of events designed to make me look good. Well, I suppose that is a problem with all autobiographies. In any event, here, without further apology, is my attempt to present an "accurate" portrayal of my career to the present day.

## Roots and Childhood

Ada Machlus and Isadore Kravitz were married in Philadelphia, PA, in 1929, the city where they were born 20 years earlier. My mom had just graduated from high school and was working in a department store at the time: dad never finished high school. Shortly after the marriage, they moved to

New York where my brother Bill was born. I was born close to three years after that in December of 1932. We're actually not sure what the family name was. When dad was born, a doctor asked my grandfather for the family name: he said Koretsky, we think. The doctor told my grandfather that you could not raise an American boy with that name. Instead, they took my grandmother's family name, which was Kravitz.

Dad hit the New York job market at the start of the Great Depression. He co-owned a gas station for a while that supposedly was stolen from him by his partner, worked as a Western Union telegram delivery boy, and later sold Wearever aluminum pots and pans after cooking meals for groups of housewives in their homes. At some point during the 1930s, dad began working for his father in the garment industry. Samuel Kravitz ran a shop in downtown New York making expensive women's coats and suits as a subcontractor for other manufacturers. My father became a "cutter," which was the most important position in the shop. Large, heavy, multiply layered rolls of expensive, mostly woolen material were delivered to contractors along with patterns or forms used for cutting the pieces to be sewn into coats and suits by the "machine operators." The patterns developed by the manufacturer's cutters were used to calculate the numbers of coats and suits to be produced from the rolls of cloth supplied to the subcontractor. Standing in front of a huge, centrally located cutting table with the unrolled layers in front of him and with a large ceiling-mounted circular saw, dad always figured out how to get many more garments from each pattern than the numbers calculated by the manufacturers cutter. These were made into coats and suits that my grandfather sold privately at reduced market prices, but at huge profit for himself. Dad saw little of the "extra" money made by his father in this way.

As the expensive hand-crafted women's garment industry slowly died after World War II, dad played more and more of a role in keeping the earnings of his father and stepbrother coming in. First, my grandfather purchased a cluster of bungalows in Far Rockaway, NY, for summer rental by city dwellers escaping the New York heat. Dad became the caretaker for these bungalows, teaching himself plumbing, electrical wiring, carpentry, and painting along the way. In fact, there was nothing that dad could not do once he set his mind to it. He invented and patented a cigarette machine that delivered one cigarette at a time for a penny and that caught slugs (fake pennies). He invented an industrial-sized distilling apparatus to recapture purified perchlorethylene from waste dry cleaning fluid when the family purchased a series of dry cleaning stores in Harlem upon the demise of the garment industry shop. Dad subscribed to *Popular Science* and other science magazines of the day. One time I remember him being fascinated with and spending endless hours exploring magnetism after reading an article on the topic. He built an early crystal radio while still in Philadelphia, and we were among the first people in our Bronx neighborhood to own a television set. One year dad had to throw out unwelcome guests who had crowded into our

living room to watch the New York Yankees in one of the first World Series to be televised and who refused to leave as the game went into extra innings. I suspect that with an education, dad would have been a great scientist or engineer, with his inquiring and agile mind, his uncanny ability to learn new things, and his knack of getting things to work.

Dad and mom together started the Bronx Chapter 85 of "The Mended Hearts" after dad's mitral valve replacement surgery in 1972. That too had an interesting history. The day dad was brought to his hospital room from intensive care, he overheard the nurses talking about a patient who was to undergo the same surgery and who was terrified at the prospect. Dad asked to be wheeled down to the patient's room, was helped to a sitting position at the foot of the bed, and said to the patient, "Hey, I had the same surgery 3 days ago, and look at me now." Apparently, that did the trick. The patient calmed down and underwent a very successful surgery. When dad's surgeon (Dr. Frater, who had been trained by Barnard) heard what he had done, he asked whether dad would be willing to start a chapter of The Mended Hearts at the hospital. Together, dad and mom gathered the necessary paperwork, and in June of 1973, Chapter 85 was chartered with dad as the president and mom as the secretary. The Mended Hearts is a national organization of former heart patients who individually visit cardiac surgery patients before and after surgery in hospital rooms and who hold regular group meetings in the hospitals as well. As dad's guest, I went to one of the meetings. Nurses, standing beside wheelchairs containing patients who were to undergo cardiac surgery in the days ahead, surrounded the room. Seated in the audience were former cardiac patients and their families. As each former patient stood up and listed the date and nature of their surgery, the faces of the waiting patients got brighter and brighter. It was positively inspirational. Dad was honored in 1979 by the Borough President of the Bronx for starting the first chapter in the borough and one of the first in New York City.

Mom was the one who raised me and my brother. She was the social chair of dad and mom's life together. Mom made the arrangements to see friends and family. Mom did the planning of summer vacations to the Catskill Mountains in New York, or later of summer vacations to Far Rockaway, or even later of vacations for the two of them to Florida. Mom was really a great organizer. I vividly remember her standing in her Brigadier General's outfit as a member of the Women's Volunteer Corps during World War II where she organized War Bond drives and collections of scrap metal and paper. She did the family finances and made sure that in the worst of times we were properly nourished and clothed. She picked the furniture and decorated the house. She wrote poetry and songs, none of which was ever published, and she played piano, although her lessons ended after her parents lost all their savings in the bank crashes of the 1920s. She also worked with and helped dad in the cleaning stores and was the mainstay of the Mended Hearts.

I remember her from my childhood as slender and glamorous, with long dark gently waving hair surrounding an angular, narrow, attractive face. She was the life of every party, dancing the evenings away and moving from table to table greeting friends and family. I remember her in later life, with white short cropped hair, not quite as slender or energetic, as the years and a reasonably hard life had taken their toll.

I was a smart kid growing up in a neighborhood and going to schools in which being smart was not appreciated. Schools did not know what to do with smart kids, so they had me skip grades, which invariably placed me in with older and bigger kids. The result was that I was in college at age 16, which was much too young to be in college. Before college I was with kids who did not associate with me because I was so young. My defenses against this were to develop a sharp tongue and quick wit and to become serious about sports. In grade school and high school, I played baseball as a catcher and basketball as a guard on neighborhood pick-up teams. In college, I played basketball for the 92nd Street "Y" team. After college, while at Sloan–Kettering for a year, I played third base in the city fast-pitch softball league. Sports was an important part of my youth, an enthusiasm that continues to the present day, when mostly I play tennis.

What I remember most about growing up in the Bronx was endless evenings sitting with friends on Mr. Hopengarten's newsstand outside the corner candy store. When chased from that perch, which happened nightly, we gathered around the corner to engage in noisy street games (Johnny on the Pony, Ring-o-levy-o, stoopball). Eventually, we were chased from those games as well. In fact, my friends and I seem to have spent an inordinate amount of our youth being moved from location to location over the neighborhood by complaints of storeowners, landlords, neighborhood residents, and the police, all of whom seemed to think we were creating disturbances. A favorite daytime game was stickball, which could be played in any of several ways. If enough kids were available, it was played with batters hitting on their own and other players spread out at positions roughly filling a narrow baseball diamond chalked out on Burke Avenue in the Bronx—a busy uphill main thoroughfare. It could also be played with a pitcher and one fielder and either an intact or split-in-half ball in the neighborhood back lot. In either case, the bats were broom handles pilfered from unsuspecting parents (since these were regularly confiscated by the police, we believed that there soon would be no intact brooms left in our neighborhood), and the balls were pink "Spaldeens," as they were called, until I eventually found out that they came from a box labeled "Spalding" in Mr. Hopengarten's store. When the Spaldeens split in half with use, they became the half balls used in backyard stickball. The police in New York seemed particularly intent on breaking up stickball games. The cry "chickie-da-cops" alerted us to throw the bat under the nearest car and gather in small groups chatting innocently. Somehow or other, the bats always were found by the relentless officers. Maybe it was the

unusual placement of groups of three or four kids around home plate, first base, and the outfield that gave us away. I have no memory whatsoever of ever doing homework. I must have done some though, since I did graduate from various schools. Try as I might, I conjure up no images of me sitting in our small first floor one-bedroom apartment, burning midnight oil preparing for exams, or even doing any reading or school work.

## College Days and Sloan-Kettering (1949–1954)

I did manage to get into college, barely passing the competitive examinations required for admission to the City College of New York (CCNY), after just making the honor roll at our neighborhood high school, Evander Childs High School in the Bronx. No one ever told me about the Bronx High School of Science or Stuyvesant High School where the brightest kids in the city went after completing eighth or ninth grades. College for me was a continuation of high school life, with evenings spent on Mr. Hopengarten's newsstand, me doing little home study, and my primary interests focused on girls and basketball (note to young folks: don't try to emulate this lifestyle—it just won't work these days).

One thing I vividly remember about college life was two summers working as a counselor in camps for handicapped children (Camp Oakhurst in Oakhurst, NJ, and Cradle Beach Camp in Angola, NY, on the shore of Lake Erie near Buffalo). I have never forgotten those young boys and girls dealing so bravely with devastating disorders such as muscular dystrophy, congenital birth defects, cerebral palsy, blindness, and epilepsy. Nor can I ever forget the way that most of the public reacted to outings with those children, looking the other way as we passed, offering us money, or hurrying by pretending not to see us or the children. Of course, some people opened their hearts to us and wanted to do something for the children. Like the time an operator of a "Dodgem Cars" attraction at an amusement park closed the ride to outsiders and gave us and the children the sole use of the ride in an environment where we would be safe. I have never seen a happier group of kids smashing into each other's cars on an amusement park ride. I am certain that the roots of my dedication to inspiring new generations of students to find solutions to neurological and psychiatric disorders are the two inspirational summers I spent working with these amazing youngsters.

I did get two A grades at CCNY. One was in basketball. The other was in Physical Chemistry, which was the toughest science course at the school and the only one I found challenging. As the end of college life approached, a difficult question loomed: What was I going to do with the rest of my life? Without much conviction, I applied to two medical schools and to be an officer in the U.S. Army Medical Corps: all three applications were rejected. Then I had a lucky break. I applied for and got a job as a Research Assistant to Dr. George Tarnowski, who had a small laboratory in the Chemotherapy Division at

Sloan–Kettering Hospital. My duties included injecting small pieces of solid tumors or Ehrlich ascites tumor cells into mice and then injecting drugs in what invariably turned out to be vain attempts to reduce the growth of the tumors. Dr. Tarnowski's laboratory adjoined another small laboratory where Dr. Lou Kaplan, a young biochemist, was studying the metabolic properties of mouse ascites tumor cells. Lou also played shortstop on the Sloan–Kettering softball team that played in the New York City Hospital League. With Lou's encouragment, I tried out for the team and ended up playing third base. Lou also encouraged me to do a research project. With Dr. Tarnowski's support, Lou's help, and the permission of the director of the chemotherapy unit (Dr. Christine Riley), I began a research project looking at amino acid metabolism in ascites tumor cells (mostly I remember breaking a lot of equipment). Once I started doing research, I was hooked. Finally, I had found something that excited me. That led to a night school course in biochemistry at CCNY, where I received an A grade, and applications to graduate programs in biological chemistry at Rutgers and the University of Michigan. Both accepted me, with the Michigan acceptance requiring that I maintain a B average in graduate school. I chose Michigan on the advice of the folks at Sloan-Kettering and quickly convinced the skeptics at Michigan that the risk was worth taking by maintaining an almost straight A average throughout my graduate career.

## Start of My Life as a Scientist (1954–1959)

When I first arrived at the University of Michigan, Biological Chemistry was a department in transition. There was an older faculty (Adam Christman was the Chair) who were close to retirement age, who taught "classical" biochemistry, and who for the most part did not run active research programs. Saul Roseman (a distinguished investigator working on complex carbohydrate biosynthesis) was an exception, but he was not based in the West Medical Building that housed most of the department. Saul did play an important role in keeping me in graduate school, though, when a dispute broke out between me and my thesis advisor about storing solutions in volumetric flasks. Jim Hogg (carbohydrate biochemistry) and Merle Mason (tryptophan metabolism) also were active in research. There was a younger, newer faculty, who had recently been hired and whose numbers continued to grow during my graduate student years. These included Armand Guarino, a purine biochemist, who became my thesis advisor; Paul Srere, a carbohydrate pathway biochemist, who became a close friend and scientific mentor (Paul was the person who originally told me "the suit joke"—see below); Halvor Christensen, who was hired as the new Chairman of the Department soon after I arrived at Michigan; Robert (Bob) Greenberg, a nucleic acid chemist; Minor J. (Judd) Coon, who worked on intermediary metabolism; Bill Lands, a lipid chemist; and several others whose names

escape me now. During my first year I received training in "classical" biochemistry: I crystallized proteolytic enzymes using the original methods and measured gas exchange with a Warburg apparatus. In seminar we debated issues like whether proteins or nucleic acids carried the genetic information. The student group was strong and cohesive, leading to many close and lasting friendships with my peers. Marshall Nirenberg, who won the Nobel Prize for cracking the genetic code a few years later while at NIH, was a few years ahead of me. Marshall and I shared an apartment on Huron Avenue for a while before he moved to NIH. Later, I heard that our house had been replaced by a church. I attach no significance to these two events. Joe Merrick, Chava Spivak, Halina Den, Milt and Sandra Schlesinger, and Usama Al-Khalidi formed my circle of friends. The qualifying exam for admission to Ph.D. candidacy was done in a novel way: one day during your second year of studies, a faculty member came up to you and said, "Your exam is now." I was advised that a good strategy for these exams was to get the faculty examiners arguing among themselves. By succeeding in doing that, I passed easily.

Armand gave me a free hand to work on whatever I desired. He hoped, of course, that it would be related to purine metabolism. I chose a project that probably was slightly larger than what he envisioned. I became interested in the question of how DNA was synthesized, which was not known at the time. What were the precursor molecules? How could I get at them? I made a few attempts to develop a cell-free system to study DNA biosynthesis by incubating 5′-deoxynucleotides, ATP, magnesium salts, and crude enzyme extracts from ascites tumor cells and other sources, but none of these worked. In order to do these experiments, I had to isolate the 5′-deoxynucleotides that I included in the incubations from DNA by hydrolysis, separating the nucleotides on ion-exchange columns. Sigma had started commercially supplying 5′-deoxynucleotides at that time, but supposedly Arthur Kornberg was buying out their entire supply. With the failure of my first experiments, I decided to try a different approach. Perhaps I could find and identify precursors if I used radioactive tracers in living cells under experimental conditions in which the cells were actively making DNA. I chose logarithmically growing *Escherichia coli*, which I knew had to be making large quantities of DNA, and added a short tracer pulse of radioactive guanine to the cultures. Periodically, I withdrew samples and separated them into acid soluble, RNA, and DNA pools to follow the radioactivity in the search for precursors. What I observed was that a large early peak of radioactivity appeared in the acid soluble pool, which was followed by a slower rise in radioactivity in the RNA fraction and a still slower and smaller rise in radioactivity in DNA. Thus, nothing particularly informative appeared regarding DNA biosynthesis; however, one very surprising result was obtained. I noticed that the counts in RNA were not stable, even though the literature of the day said that once synthesized RNA was stable and did

not turn over. My counts went down by about 10% after the peak incorporation of radioactivity, and thereafter, the radioactivity in the RNA pool remained stable. I showed these results to many people, but everyone said that it was an artifact—that I was doing something wrong—that I shouldn't do anything with the results. Two years later, Astrachan and Volkin did a similar experiment using an only slightly different experimental system (bacteriophage-infected *E. coli*) and discovered messenger RNA. I've kept that notebook, with the original results, to remind me that when I talk to students about careers in science I should tell them not to necessarily listen to older and wiser advisors—at least not all the time.

My thesis research actually involved the role of inorganic phosphate in regulating the choice of pathways through which glucose would be metabolized in ascites tumor cells. The topic was selected after I accidentally discovered an inhibition by inorganic phosphate of the enzyme glucose-6-phosphate dehydrogenase, the first enzyme in the pentose-phosphate "shunt" pathway of metabolism. Extended discussions with Paul Srere helped sharpen the definition of the problem. The thesis described effects of inorganic phosphate on the choice of pathways of carbohydrate metabolism using (1) crude tumor cell extracts; (2) a reconstructed enzyme system in which I isolated and purified the rate-limiting enzymes in each of the pathways, combined them in amounts present in tissue extracts, and partially duplicated the effects I observed in the tissue extracts; and (3) intact tumor cells. This work led to one publication in *Science* and left me, once again, not knowing where I would go next. It is interesting that as I look back now at my thesis, I discuss "intracellular control factors" as important "compounds capable of governing the metabolic rates of various intracellular enzymic pathways." I also pointed out that multiple factors must be involved in regulating pathways of metabolism. Thus, from my earliest published work, I was interested in regulatory factors and their roles in pathway choice. As a budding biochemist, I focused on the roles such factors serve in choosing between metabolic pathways in a complex intracellular milieu. As a neuroscientist and now a neuroethologist, I have focused on extracellular regulatory factors (neurotransmitters and neurohormones), asking how they work at a cellular level (harking back to my biochemist days) and how they are involved in pathway choice and assembling patterns of behavior at an organismic level. My interest in the nervous system began in graduate school also, via endless arguments with philosophy graduate students about whether we ever could understand how nervous systems worked through biochemical or physiological studies.

What was I to do next though? Once again, Luck (now capitalized, since it seems to have played such a major role in my career) interceded. As I was starting to write up my thesis studies, Earl Stadtman, a distinguished biochemist from NIH, delivered a seminar in our department. I was so impressed with the beauty of his talk that I went up afterwards and asked

if he had any postdoctoral openings. Earl said that one had just opened up and why didn't I apply. I did and was accepted.

## A Year at NIH and an Offer from Steve (1959–1960)

During my last year of graduate study, I met and married Kathryn Anne Frakes, a lovely, lively, highly intelligent redhead who has been the love of my life, my lifelong companion, the mother of my two wonderful children, and my best friend. Immediately after we married, Kathryn and I, driving a 1950 Chevy sedan pulling a U-Haul van, headed to Bethesda, MD, and the start of postdoctoral studies in the Stadtman laboratory. The evening we arrived, the Stadtman's were having a party, to which we were invited. Immediately upon entering the Stadtman house, Kathryn was asked to dance and was whisked away by a distinguished European biochemist, leaving me to hang up our coats. On returning to the party, I noticed this distinguished gentleman (d.g.) sliding his hand up and down my new wife's back. Not knowing what to do on this my first evening in a new environment with my new wife, I cut in, much to Kathryn's relief. At that point the d.g. said, "I don't blame you." Thus began an interesting year at NIH.

Almost immediately after my arrival at NIH, Earl Stadman left on sabbatical to work with his friend and sometime competitor, Fyodor Lynen. That left P. Roy Vagelos in charge of the laboratory, and Roy and his wife Diana soon became wonderful friends of ours. In the Stadtman/Vagelos group I began work on the metabolism of the opium alkaloids. I had a vague notion that I was ultimately going to end up working in the nervous system and had developed a plan to move in that direction. The plan involved (1) learning how morphine and related alkaloids were synthesized and metabolized in plants as first steps toward learning how they functioned in the brain and (2) doing two additional postdoctoral stints after I finished my studies in the Stadtman laboratory with investigators working directly with nervous tissues. One of these postdocs was to be with David Nachmanson at Columbia University to learn how synapses worked. The second was to be with Oliver Lowry at Washington University in St. Louis, MO, to learn the elegant micromethods I felt would be required to study the biochemistry of single nerve cells. To the biochemists, Nachmanson was a martyr who was continually under attack from neurophysiologists because he had shown convincingly that their theories about how neurotransmission and the conduction of nerve impulses worked were wrong. Nachmanson believed that acetylcholine was involved both in transmission *and* in conduction, but he believed that the process did not involve the release of acetylcholine from presynaptic terminals or from any other sites. Instead, he believed that acetylcholine was synthesized and degraded within nerve cell membranes in a cyclical fashion and that this cycle generated *all* of the electrical signals

recorded by neurophysiologists. Once again, Luck played her hand: none of these plans for postdoctoral training materialized.

For my studies on the biosynthesis of morphine alkaloids at the NIH, I had a field of opium poppies grown for me by the U.S. Department of Agriculture in Beltsville, MD. I also accumulated a collection of giant bottles of freeze-dried samples of the mold *Claviceps purpurea* for studies on the ergot alkaloids. I used leaves and roots of the poppy plants to study the biosynthesis of the opium alkaloids, but never began my planned studies with the mold samples. I was amused to hear, though, that about a year after I left the NIH, decontamination people in full body suits and masks were called upon to remove the harmless purple mold samples that I had left in the cold-room.

The move from the NIH to Harvard Medical School (HMS) came about as a result of a phone conversation between Steve Kuffler and Roy Vagelos. Steve had just moved from The Wilmer Institute at Johns Hopkins University to the Department of Pharmacology at HMS with Dave Hubel, Torsten Wiesel, Ed Furshpan, Dave Potter, and the ever-loyal electronics expert, Bob Bosler. Together they formed the Neurophysiology Laboratory in the Department of Pharmacology, with Steve as full Professor and everyone else in junior roles. Steve and Dave Potter already had begun a project aimed at identifying the inhibitory transmitter compound at crustacean neuromuscular junctions, with a biochemist colleague, Akira Kaji. Kaji left the project when the group moved to Harvard, and Steve began searching for a biochemist to continue this work. Steve also had begun to develop the philosophy that understanding the nervous system would take the combined efforts of investigators from many disciplines, including neurophysiologists, anatomists, and biochemists. In that vein, Steve was searching for a biochemist. Steve had obtained Roy's name from colleagues at NIH, and the phone call was to ask whether Roy was interested in joining the new group at Harvard. Roy said that he wasn't interested, but there was a guy in the group who kept giving journal club seminars on neurochemical topics and that he might be interested in the position. That led to a phone call to me from Steve, a chat at NIH when Steve was visiting, and an invitation to come to Boston to look at the job.

## Discovery, Creation, and Political Activism (1960–1970)

### *A Visit to Boston and a Decision*

A major snow storm was predicted for the day of my visit to Boston. I had prepared for the trip by reading, or trying to read, some of Steve's papers, which were full of incomprehensible squiggles, unfamiliar abbreviations, and cartoons. My biochemist colleagues gave me a list of things I should

request in negotiating for the position. These included a salary of around $20,000; at least 1000 square feet of my own research space; $20,000 in startup money; and, above all, a position in Biochemistry and not in Pharmacology, where Steve's unit was located. None of the people I talked to before the visit had heard of Steve or any members of the group, and therefore, they urged great caution on my part in this non-biochemical environment. The snow storm hadn't yet started when I arrived in Boston and was greeted at the medical school by Dave Potter and Ed Furshpan, as Steve was otherwise engaged. Dave vigorously pumped my hand up and down and took long, striding steps around the office as he enthusiastically described the project of trying to identify the inhibitory transmitter compound at crustacean junctions. Ed, by contrast, gave the impression of someone trying to climb the walls and escape even while he told me about his Mauthner cell work. After this conversation, Dave took me down the hall to meet Dave Hubel and Torsten Wiesel. They too tried to explain their work to me, but to no avail. They asked what I was interested in though, and I told them that, among other things, I wanted to explore the biochemical basis of learning and memory. I noticed their sideway glances at each other as I talked about my plans. Then came the visit with Steve: I was completely unprepared for what followed next.

Steve patiently listened to my list of requirements. Then quietly, one by one, he dismissed them. Surprisingly, I wasn't the least bit offended by this. In later years I came to realize that Steve was the only person I'd ever known who could fire someone and have them walk out of his office with a smile on their face. Steve said that I didn't want a position in Biochemistry, because then I'd have to teach over there. Since I was only one year past my Ph.D., he offered me an Instructor's position in Pharmacology at a salary only slightly higher than the amount I was earning as an NIH postdoc (not very much, so I negotiated that up a little bit). "Space" he said, "you'll share with us." The most compelling argument for taking the position though was what Steve said next. "What you really want is the opportunity to see whether you're any good as a scientist. I can offer you five years of research support on an NIH Program Project Grant, and am happy to purchase any equipment you need. All I require is that you work on the nervous system." Of course, Steve also knew that if I had any sense, I'd join them on the project trying to identify the inhibitory transmitter compound at crustacean neuromuscular junctions.

After the meeting with Steve, I dropped in on my friend Howard Goldfine, then in the Microbiology Department. On our way back to his house in Cambridge, we got involved in a wild snowball fight with dozens of students who came pouring out of the freshman dormitories in Harvard Yard. Since I came to Boston without snow gear, I ended up thoroughly soaked by this diversion. I caught the last train leaving Boston before the storm closed down South Station, and on a long slow trip to Washington,

D.C., I had time to think about the offer from Steve. To this day, I'm not certain what clinched my decision to come to HMS. It just seemed to make sense. Here were a group of people who seemed to know a lot about the nervous system, and here was a golden opportunity to find out whether I was any good in the laboratory. Probably most important, though, was that I really liked the people I had just met and felt that this was a place I might fit in, learn a lot, and even serve an important role.

## Discovery: GABA and Procion Yellow

### *GABA as a Transmitter Compound*

Immediately after our move to Boston, Steve, Dave Potter, Ed Furspan, Bob Bosler, and Joseph Dudel (who was visiting with Steve at the time) packed up the laboratory and their families and headed to the Marine Biological Laboratory (MBL) in Woods Hole, MA, for the summer. Steve was the Director of a Training Program in Neurophysiology at the MBL that was the forerunner of the famous biophysics and neurobiology courses of later years at that Institution. Steve invited me to join the group, and somewhat reluctantly, Kathryn and I repacked our recently unpacked suitcases and headed to Cape Cod for a month. It was not an easy place to do biochemistry, with Steve's children cramming foul smelling bait in the same freezers and refrigerators in which I was trying to store tissue samples and reagents. Steve's laboratory also contained essentially no biochemical equipment. Still, the ambiance and environment were great, and the firm bonding between Steve and his "boys" (the academic world of the 1960s was very much a male-dominated world—it still is today, but fortunately things are getting better) that began during those early summers at the MBL ultimately previewed the creation of the first Neurobiology Department in the world.

The first project I worked on that summer involved a peptide as a possible neurotransmitter. Frank Belamarich (Boston University), Ian Cooke (Harvard), and Dave Potter working independently had shown that aqueous extracts of the pericardial organs of crustaceans (a crustacean nerve ring surrounding the heart, originally described by Alexandrowicz and later extensively studied by Don Maynard) contained a potent cardioexcitatory activity that was destroyed by proteolytic enzymes. With my biochemical background and supposed ability to purify proteins, this seemed a good starting project for me in the Kuffler group. Unfortunately, the peptide proved difficult to purify, and all purification steps I tried resulted in a complete loss of physiological activity. It was 25 years later (a little late to be pioneers in the field of peptides as transmitters) that we finally succeeded in purifying the peptide. Barry Trimmer, then a postdoctoral Fellow in my laboratory, used HPLC columns to isolate and sequence two FMRFamide-related peptides

that accounted for most of the biological activity (TNRNFLRFamide and SDRNFLRFamide).

*GABA is Not a Transmitter Compound?*

In 1960 and 1961, Jack Eccles, David Curtis, Ernst Florey, Hugh McClellen, and other investigators proclaimed at two international congresses that GABA was not a transmitter compound in either vertebrate or invertebrate nervous systems. Florey's argument rested on his inability to find GABA in crustacean nervous tissues, while Eccles reported that there were significant differences between normal inhibitory mechanisms in the vertebrate spinal cord and the actions of externally applied GABA. This despite the fact that Florey was the first to suggest, *in print*, that GABA was a transmitter compound. He made the suggestion based on (1) pharmacological studies showing that GABA inhibited the firing of crustacean stretch receptor neurons (but many other substances also inhibited the firing of these cells) and (2) experiments carried out with Bazemore and Elliott showing that GABA contributed the bulk of the activity that blocked the firing of crustacean stretch receptor neurons in an extract from the vertebrate central nervous system (CNS) called Factor I. During the same period of time in which Florey first proclaimed that GABA was a transmitter compound and then that it was not, an outstanding series of neurophysiological studies appeared defining the ionic mechanism underlying inhibition in crustacean tissues and comparing that mechanism to the actions of bath-applied GABA. These studies by Fatt and Katz, Boistel and Fatt, Furshpan and Potter, Kuffler and Edwards, and Dudel and Kuffler demonstrated that the actions of GABA were identical to those of the natural inhibitory transmitter compound in crustacean tissues. Instead of claiming that GABA was a transmitter compound, however, this group of distinguished scientists cautioned that a number of essential experiments were missing and had to be done before GABA could be considered a transmitter compound. Of course, all these investigators *suspected* that GABA was a transmitter compound, but they were careful not to say so *in print*.

At the end of the summer, I began work on the GABA project. Despite claims to the contrary by Florey at both international congresses, Dave and Steve already had strong evidence that GABA was present in crustacean tissues. To demonstrate this, they dissected central and peripheral nervous tissues from 500 lobsters. They used acid extracts from these tissues in order to (1) separate physiologically active compounds by hanging curtain electrophoresis, (2) subdivide bioactive fractions using preparative paper chromatography, and (3) crystallize several of the active substances from the chromatograms. One of the compounds obtained in this way was GABA, and it represented about 30% of the inhibitory activity found in the original crude extracts. These procedures demonstrated convincingly that

GABA was present in central and peripheral nervous tissues of lobsters. To further confirm these observations, I felt it important to demonstrate that GABA actually was synthesized from glutamic acid in crustacean peripheral and central nervous tissues. This too flew in the face of published results, as Florey and Chapman reported that glutamic decarboxylase, the enzyme forming GABA from glutamate, was not present in crustacean tissues. Using radioactive glutamate labeled with $C^{14}$ at different positions in the molecule, I showed that a particulate enzyme fraction from crustacean nervous tissues would convert glutamate to GABA and, as in vertebrates, that the mechanism involved removal of the carboxyl group as $CO_2$.

When I originally arrived in Boston, I carried a test tube of the organism *Pseudomonas fluorescens* (ATCC 13430), which had been grown on GABA as a sole carbon source, in my pocket. To grow on this unusual amino acid, high levels of a pair of enzymes that metabolized GABA were produced by this particular strain of the organism.

1. *GABA/glutamic transaminase*: GABA + α-ketoglutaric acid → glutamic acid + succinic semialdehyde
2. *Succinic semialdehyde dehydrogenase*: succinic semialdehyde + TPN → succinic acid + TPNH

In 1959, Jakoby and Scott had demonstrated that these enzymes offered the possibility of a rapid, sensitive, highly specific assay for GABA by measuring the amount of reduced pyridine nucleotide (TPNH) produced when GABA was metabolized through both steps (Jakoby and Scott, *J Biol Chem* 1959;234:937–940). Just before leaving the NIH, I visited the Jakoby laboratory to collect the culture. I knew that the cumbersome assay being used by Dave Potter to separate and identify GABA and other physiologically active compounds would have to be replaced by a faster, more sensitive, quantitative procedure for measuring GABA, and the enzyme assay offered that possibility. Our next step, therefore, which involved me, Dave, and Nico van Gelder (a second biochemist who arrived at HMS when I did), was to use the Jakoby and Scott enzyme assay to measure levels of GABA in peripheral axons. For our first studies using this procedure, we analyzed mixed nerve bundles containing excitatory, inhibitory, and sensory axons; then smaller bundles containing only excitatory and inhibitory axons; and finally, single inhibitory and excitatory axons. The relative concentrations of GABA in these tissues increased dramatically as we came closer to pure inhibitory axons, finally reaching the surprisingly high concentration of 0.1 *M* in single inhibitory axon extracts.

We had fun during those early days preparing the enzymes used for the GABA assay (no kits were available), usually at the expense of new lab members. First, we grew up huge quantities of bacteria. Then, to extract proteins from the bacteria, we used the infamous "French Press." This device allowed

one to subject concentrated suspensions of bacteria to thousands of pounds of pressure, then to drop the pressure to one atmosphere, thereby exploding the bacteria and yielding concentrated, highly active crude enzyme solutions. As each new person joined the Kuffler lab, we invited him or her to assist us in preparing our enzyme extracts. Their role would be to pump the handle of the enormous jack used to compress a plunger in the specially constructed steel cell containing the bacterial suspension. Of course, it got harder and harder to pump as the pressure within the cell grew higher, and when we released the contents of the cell to atmospheric pressure via a small valve at the bottom of the cell, the person manning the handle had to pump furiously to maintain the required high pressure within the cell. Our "volunteers" invariably ended up red-faced and exhausted. No one ever volunteered a second time.

During those early years in Boston, I learned many neurophysiological techniques: how to identify and dissect single axons (my first single excitatory and inhibitory axon dissections took 6 hr; by the end of several months they took about 20 min); how to use a physiological rig and record from single muscle fibers with intracellular electrodes; how to set up neuromuscular preparations for bioassays and for release experiments; and a little later in the mid-1960s, how to find and identify CNS neurons, a technique pioneered by Masanori Otsuka at the start of his sabbatical visit with us from Tokyo Medical and Dental University. Masanori joined the laboratory shortly after the completion of the experiments demonstrating the selective localization of GABA in crustacean inhibitory axons. In a set of elegant studies, he combined the physiological identification of neuronal cell bodies to map neuron position in central ganglia, with single cell biochemistry. In so doing, he generated the first detailed maps of the positions of physiologically identified neurons in an invertebrate central ganglion. When Masanori presented these results to a packed meeting room at a Federation of American Societies for Experimental Biology (FASEB) meeting (before the days of the Society for Neuroscience), 5 min of applause followed his talk, something I never had heard before at a national meeting.

Only two substances, acetylcholine and norepinephrine, were recognized as transmitter compounds in the mid-1960s. We knew that by adding a third compound to that list we would be doing something of great importance. We also knew that the most essential experiment, the release experiment, remained to be done. We had to show that GABA was released by inhibitory and not by excitatory nerve stimulation. We also understood that to really be a transmitter compound, enough GABA had to be released to exactly duplicate the effects of inhibitory nerve stimulation. That particular requirement, however, had not at the time and still has not been satisfied for any transmitter compound at any junction. Moreover, in studies with Les Iversen (a neuropharmacologist/biochemist who had been sent to us by Julius Axelrod and Arnold Burgen) and with Paula Orkand (an anatomist), we had

shown that a GABA uptake system existed in crustacean neuromuscular preparations. With no way to inhibit the uptake system, other than by omitting $Na^+$ from the bathing medium which would block conduction, any GABA collected by us only represented the overflow from the uptake system.

Dave Potter and I had made a few early attempts to demonstrate GABA release, but found ourselves searching for GABA at the limits of detection of the enzyme assay, even at its most sensitive. Masanori also made several attempts to demonstrate the release of GABA, using radioactive GABA that was taken up into muscles, but he too was working at the limits of detection of his method. We speculated that larger muscles containing greater numbers of nerve terminals would be required to bring us over the threshold of detection of GABA in saline superfusing muscle preparations. Dave and I were using the opener muscle of the dactyl (the moveable finger) of the walking leg for our early experiments (the same preparation used for bioassay), because these were easy to dissect with their innervation intact and because the surrounding exoskeleton formed a chamber suitable for superfusion with minimal volumes of saline. In the search for larger muscles, we went to the much larger opener muscles in the crusher claws of lobsters (we called them the "big openers"). By that time Dave and Steve had turned their attention elsewhere, leaving Masanori, Les, Zach Hall (my first graduate student), and myself the task of trying to complete the release experiments.

These were labor-intensive, long-lasting experiments in which we divided the many tasks involved between the four of us. First, there was a difficult dissection, requiring cutting through the tough exoskeleton surrounding the claw without damaging the muscle and cleaning the muscle surface of as much connective tissue and clotted hemolymph (lobster blood) as possible without damaging the nerves innervating the preparation. Next, the preparation had to be set up for superfusion, the excitatory and inhibitory nerves drawn into suction electrodes, and intracellular microelectrodes inserted into muscle fibers to record synaptic responses. The tissue had to be superfused with saline for 4 hr to lower a background washout of GABA to low and stable values. Then in 25-min time bins we stimulated excitatory and inhibitory nerves while superfusing muscle preparation with saline containing or lacking calcium (to block transmitter release). Even under these optimized conditions, the amounts of GABA released turned out to be very small. They were in the range of $10^{-10}$ mol of GABA for a 25-min period of continual stimulation of an inhibitory axon. An elaborate ion-exchange procedure quantitatively recovered these tiny amounts of GABA from a multimillion-fold excess of salts in the saline collected during the superfusion periods. Finally, the enzyme assay at its highest sensitivity was used to measure the amount of GABA in each sample. It was rare that everything worked in a single experiment, so we had to carry out enough of these difficult experiments to convince ourselves that GABA was indeed released by inhibitory nerve stimulation.

While my three colleagues literally were up in the air, I completed the experiment that unequivocally demonstrated the transmitter role of GABA. Masanori was on his way to Japan, Les was on his way to England, and Zach was on his way to California. The four of us began the experiment together, but Masanori, Les, and Zach left for the airport during the experiment, leaving me to complete the final analysis. Fortunately, the experiment worked. With no email, "snail mail" and phone calls announced the results: we now had in hand the final crucial piece of evidence required to show that GABA was a transmitter compound.

*How Was Our "Discovery" Greeted?*

Soon after that at the MBL, I gave my first major talk on GABA as a transmitter compound. The first person to stand up after the talk was David Nachmanson who said, "Well, we don't know what that little bit of an amino acid that you see being released is when you stimulate a nerve, but it certainly is not a chemical transmitter compound, because we all know that transmission is electrical." Les had a similar experience when he presented the results at a Royal Society Meeting, where someone in the audience took issue with him calling GABA a transmitter compound. It couldn't be a transmitter because it was released from a neuromuscular junction and not a synapse. Luckily, Steve came to my defense at the MBL and Bernard Katz to Les's defense in England. Even 20 years later, in May 1985 at the inaugural meeting of the Merck Sharpe and Dohme Neuroscience Center in England, we didn't fare much better. Les had asked Kresimer Krnjevic to give a history of GABA as a transmitter for the meeting. His history divided the story of GABA into various ages. The 1960s, when we thought we had shown that GABA was a transmitter, were considered the Dark Ages by Krnjevic. The Rennaissance, according to him, wasn't until the 1970s, when investigators finally began to believe that GABA might be an inhibitory transmitter compound in the vertebrate CNS. It was one of my first encounters with a higher vertebrate chauvinism, that unfortunately has come more and more to dominate neuroscience research *and neuroscience funding* in this country. Even today, it is difficult to find in most textbooks of neuroscience mention of the crustacean story demonstrating the transmitter role of GABA.

To complete the story of GABA as a transmitter compound, we sought an explanation for the selective accumulation of GABA in inhibitory neurons. We carried out these studies at about the same time as the release experiments. With Deric Bownds (a postdoctoral Fellow from the Wald laboratory), Perry Molinoff (a medical student), and Zach Hall, we worked out the pathway of GABA metabolism in crustacean tissues, characterized the lobster enzymes, scaled down our assays for these enzymes to the point where we could measure activity in single axons, and quantitatively measured the levels of enzymes and substrates for the GABA pathway in single excitatory

and inhibitory axon extracts. Deric even ran microgel electrophoresis of the extracts of single axons to demonstrate that no decarboxylase activity was detectable in excitatory axon extracts. The results of the single axon experiments offered an explanation for the selective accumulation of GABA in inhibitory neurons and allowed a suggestion of why GABA accumulated to an 0.1 *M* concentration in inhibitory axons. The data showed that the synthetic enzyme glutamic decarboxylase was found only in inhibitory axons, but the degradative enzymes, the transaminase and dehydrogenase, were found in both excitatory and inhibitory axons. Without decarboxylase, excitatory axons could not accumulate GABA. The units of enzyme activity showed that inhibitory axons could synthesize more GABA than they could destroy, thereby allowing GABA to accumulate. At 0.1 *M* levels of GABA, however, product inhibition of the decarboxylase reduced the synthetic capability to the levels of the degradative capability. Thus, 0.1 *M* GABA, which was the final concentration in axons, represented a steady state in which synthesis was balanced by destruction.

Of course, some people did appreciate our work on GABA. With help from Jack Eccles and others, I was nominated for and became a tenured Professor at HMS only 9 years after my arrival as an Instructor. Les became the director of an MRC unit in Oxford, and Masanori became the youngest professor in Japan. Zach went on to a postdoctoral position at Stanford and to his own distinguished career.

*Procion Yellow and Neuronal Geometry*

The other major research story from our laboratory in the 1960s began when Tony Stretton (a postdoctoral Fellow sent to us by Sydney Brenner) and I began our studies of neuronal geometry. Tony and I were interested in whether identified cells in lobster ganglia always had the same geometrical shape. The question arose from Tony's background in molecular genetics and the two of us starting to ask questions such as "were the shapes of neurons genetically specified." The use of lobster central ganglia to address this question derived directly from Masanori Otsuka's maps showing that the cell bodies of identified neurons were in pretty much the same positions from ganglion to ganglion and from animal to animal. At the time, a method developed by Ed Furshpan and Jaime Alvarez (a postdoctoral Fellow from Argentina) seemed to offer an ideal tool with which to address the question. To try to determine where particular synaptic inputs were localized on Mauthner neurons in fish brains, Ed and Jaime attempted to localize their recording electrodes through the use of immobilized dyes. They had solved many technical problems around injecting dyes into neurons and in processing tissues in ways that allowed them to localize the sites of injection. In addition, they had accumulated an extensive collection of dyes in their search for the appropriate substance to inject into the Mauthner cells. They

generously shared this knowledge with us and allowed us access to their dye collection. Among Ed and Jaime's dyes was a Procion dye, and this worked best of all the substances we tested. Still their dye did not fully stain the neuropil processes of the neurons we injected. A visit to Imperial Chemicals in Providence, RI, the manufacturer of Procion dyes that were used to stain fabrics, provided us with 120 Procion-related dyes. We tested all of these dyes by injection into lobster central ganglia. Only Procion Yellow showed the features we required. It was highly soluble, readily releasable from microelectrodes, completely filled cells and their processes, and survived fixation and dehydration. In addition, and most importantly, it was fluorescent, which enhanced our ability to detect the dye in the fine branches of neurons and in nerve terminals, thus allowing us to easily localize the dye in tissue sections. Using Procion Yellow, we injected over 100 physiologically identified neurons, processed and sectioned the ganglia containing these neurons, and reconstructed cell shapes from these injections.

I vividly remember Edith Maier (our superb research assistant) completing the first reconstructions of a pair of identical cells from different animals, with Tony and I hovering over her shoulder. As each data point from the photographs of the serial sections was hand drawn onto the reconstructions (no computer programs existed for reconstruction of neurons in those days), it became clearer and clearer that the two cells had close to the same morphological shape in the two animals. In great excitement, Tony and I ran down the hallway telling everyone the results. Our ardor was cooled, however, by the responses we received, ranging from "so what?" to what did you expect—after all, Purkinje cells all have pretty much the same shape too." At first, only Hubel and Wiesel recognized the potential of the method, and within days they were attempting to fill vertebrate CNS neurons with the dye. Procion Yellow had a short lifetime, being replaced within a few years by the much more fluorescent and easier to obtain Lucifer Yellow. However, Tony and I had the joy of developing a technology that we knew would allow investigators to unravel the morphology of complex synaptic regions, a task that Bullock and Horridge had declared to be impossible just a few years earlier in their monumental work "Structure and Function in the Nervous System of Invertebrates." Our colleagues from the Biochemistry Department wondered how two good biochemists like us could waste our time on such a mundane anatomical problem.

## Creation: A Department of Neurobiology at HMS

### *The Neurophysiology Laboratory in the Department of Pharmacology at HMS*

Though science was first and foremost in our lives at HMS during the early 1960s, there was much more. Steve was Dad to his "boys," and Thanksgiving

dinners with him, Phyllis, and the Kuffler kids (Susy, Damien, Genie, and Julian) and regular Sunday morning phone calls were part of the routine of our lives. Steve never returned from a trip without greetings for each of us from colleagues. He was a notorious punster, and at one time was restricted to one pun a day (a rule he regularly broke). Probably the most chaotic time of the year, though, was the end of November, when the design for the annual Christmas card had to be created. All work stopped as we brainstormed the topical theme for the year, after which all activities in and around the photography lab stopped while photos were taken of everyone in the department, and the card was constructed, photographed, printed, addressed to colleagues all over the world, and sent out.

*The Parties*

The legendary Christmas parties began with a "social hour" and party games and continued with a huge sit-down meal cooked by Theresa (our lab assistant for many years) and her family in the jam-packed lunchroom. After dinner, there was the "suit joke" and the student skit satirizing the faculty. The suit joke is an action joke that I told over at least a 30-year period at Christmas parties and that had occasional performances at restaurants in San Francisco, at places where I gave seminars, and at international meetings in Norway and England. Steve and Roy Vagelos were great fans of the joke. It's hard to describe the joke other than to say that it was told in an ethnic (Jewish) dialect and involved extensive, rather ridiculous-looking body contortions around a new suit that didn't fit properly. The following of the suit joke was enormous. Children who grew up hearing it over the years at departmental Christmas parties would correct me if I changed even a single word. After the entertainment, the tables and chairs were removed from the lunchroom and the dancing started. Lab spring picnics and communal meals at Woods Hole in the summers complemented the "eating scene." Steve was a visible and active presence at these events, and almost all of our children were tumbled upside down over his shoulder at least a few times over the years. Once a month "evening meetings" were held at which lab groups took turns preparing dinner for the department and presenting their latest experiments in detail. While these ended in long evenings, it was an important way to keep abreast of what was happening in an ever-growing department. Almost daily seminars were held over lunch, and the week concluded with a departmental beer hour (with elaborate snacks) on Friday afternoons.

*The Lunchtime Seminars*

I don't remember when the scheduling of talks at lunchtime began. When we arrived at HMS, all medical school departmental seminars were held at 4:00 PM, usually with tea beforehand. Our seminars probably grew out of the elaborate, highly ritualized lunches we ate together in the Pharmacology

Department lunchroom (much to the amusement of the rest of Pharmacology). I suspect they started by our first asking guests to join in the repast and then asking them to tell us what they were doing. The logic of having seminars at lunchtime was "well you have to eat lunch anyhow, and we all eat together, so why not listen to talks at the same time." Sometimes, for days on end, we had lunchtime seminars. No notices were sent out announcing these seminars, and only rarely were they formally scheduled in advance. Instead, they were written on a calendar hanging on the lunchroom door, which therefore had to be checked daily to see whether there was a talk that day. Steve's wide circle of friends regarded a stop in Boston as an essential part of any trip. As each of us became prominent in our fields, we too had regular visitors. Essentially, all visitors were asked to tell us about their latest experiments over lunch. At first this caught visitors by surprise. Pleading that they had not brought slides, we said, "it's OK, just go to the board and tell us what you're doing—it's really very informal." On second visits though, friends showed up with sets of slides in their pockets and talks prepared, just in case.

The entire department turned out for seminars, cramming into the small lunchroom that was the hub of so many departmental activities. Great scurrying around preparing lunches preceded the talks, which started around 12:15 PM (the origin of the 12:15 start time of the much more formal departmental seminars today). The seminar speakers were introduced by their hosts and then the trial began. Speakers were lucky to show one or two slides (if they had brought slides) or to get through the introduction to their presentation before the questions started flying. At times, it seemed as if every detail of every slide was being questioned, which had to be frustrating for the speakers, but was exciting for us. We shared an overwhelming desire to really know and understand what was being done, why it was being done, and whether the results supported the conclusions. I don't believe it was arrogance on our part, although I suspect it bordered on rudeness. The discussions could go on for hours, until we, or the visitors, exhausted by the ordeal, called for closure. On one visit to the department, Paul Greengard, who had a biochemistry seminar scheduled for 4:00 PM was asked to deliver a lunchtime seminar. An exhausted Paul barely finished the session when it was time for him to deliver his biochemistry seminar (which we all attended, of course).

More often than not, the seminars were the highlights of our days, and they were exhilarating. It's the way we learned about the breadth of a newly emerging field. We were treated to Bernard Katz delivering a 3-hr Saturday morning discourse on synaptic transmission, and we were visited and lectured to by many past, present, and soon-to-be giants of the early days of neurobiology. A few of the large pool of visitors included Seymour Benzer, Sydney Brenner, Ted Bullock, Jose del Castillo, Francis Crick, Jack Eccles, W. Feldberg, TP Feng, Norm Geschwind, Paul Greengard, S. (Hagi)

Hagiwara, Eric Kandel, Vernon Mountcastle, Walle Nauta, Rami Rahamimoff, Miriam (Mica) Salpeter, Gordon Shepherd, Ladislav Tauc, Pat Wall, and Victor Whitaker.

*Teaching*

Under the leadership of Ed Furshpan and Dave Potter, our department always has had a serious, dedicated commitment to outstanding instruction. The neurobiology block of the medical school curriculum consistently received rave reviews from medical students. On occasion, this led to notice by the greater medical community as well. In the late1960s, we were visited by the President of the American Academy of Neurology wondering why so many young doctors from HMS were turning toward neurology. In the early years, Ed and Dave headed off to Woods Hole several weeks before the scheduled start of the neuro-block of teaching for medical students (Area III in those days) to prepare their lectures. The lectures were not memorized, but instead were an elegantly crafted, carefully thought through, and argued out system of presenting neurophysiology in a comprehensive and comprehendible manner, with each lecture building on an earlier one and leading logically to the next. To do this, Ed and Dave stood in front of and "rehearsed" each other, thrashing out the best ways to cover the material and examining the current literature to construct their lectures. The result was some of the clearest and best lectures ever presented at HMS and a system of teaching and learning that the medical students loved.

I joined Ed and Dave at Woods Hole for these rehearsals and added my few "biochemistry of synaptic transmission" lectures to their elegant set of neurophysiology lectures. A few well-placed "jokes" also were added to the lectures (probably because Jack Diamond, a visiting colleague from Canada, and I joined Ed and Dave in Woods Hole), and these too built on each other and showed up in multiple lectures. Presentations by Dave Hubel and Torsten Wiesel rounded out the Area III lecture set. Steve lectured for one or two of the early years, but was not invited to participate in future years because his presentations were not considered clear enough (we suspected that Steve did this on purpose). The popular Kuffler and Nicholls textbook *From Neuron to Brain* was heavily based on the spectacular teaching system originally devised by Ed and Dave. On top of all of that, Ed and Dave memorized the names of the medical students from the class photos sent to us each fall and surprised and delighted many a medical student of that era by calling them by their first names as they walked in the door for the first class sessions. My dedication to teaching, initially inspired by Ed and Dave, began in those early days and continued throughout my career with courses at Harvard for advanced undergraduates and graduate students in Synaptic Chemistry and the Neurobiology of Disease (which continues to the present) and with national courses such as the MBL Neurobiology Course

and the Neurobiology of Disease Teaching Workshops at the Society for Neuroscience annual meeting.

*A New Department and a New Direction for HMS*

The Pharmacology and Physiology departments were without Chairs in 1966. Our Neurophysiology Laboratory based in the Department of Pharmacology was in full bloom under Steve's leadership, with major, fundamental research discoveries being made by all members of the original group. Thus, it was reasonable for Dean Bob Ebert to turn to Steve and ask which of the two departments he would like to take over. This began a round of discussions within our group, most of which bogged down on two issues. The first was that each of the existing departments already included substantial numbers of tenured and non-tenured faculty, some of whom were carrying out distinguished research, but others of whom were not. In joining either of the existing departments, there would be a major expansion of our group, and we would lose the coherence that was the hallmark of our department and its greatest strength. Steve's style of running the department as a family also would be lost, and we would become like all other Physiology and Pharmacology departments in the country. The second concern was that we would be responsible for the teaching of either Physiology and Pharmacology, and none of us had an interest in doing that. Teaching was a major part of our lives, but we were teaching in areas we were expert in, which undoubtedly contributed to the outstanding quality of the courses we offered. Finally, we all agreed. We wanted our own department and, after prolonged discussion, decided that "neurobiology" was the name we wanted assigned to the department. We recognized that we might run into substantial opposition in the faculty to this notion. Most departments in most medical schools in the country were based on a set of methodologies, such as biochemistry and biochemical methods, anatomy, pharmacology, physiology and their methods. We were requesting something different. What we proposed would create a department based on understanding how the brain functioned, using whatever methodologies were required to do that. Steve's view was that one used whatever tools were required to understand the nervous system. Hence, the new department would include neurophysiologists, biochemists, anatomists, and, eventually, molecular biologists and geneticists.

The issue came before the faculty on June 17, 1966. The Dean strongly supported the concept and had done so in a document that was sent to the faculty prior to the meeting. Then a most interesting discussion ensued, which was mostly a turf war. Jordi Folch-Pi, a well-known lipid biochemist who had built a Neurochemistry Unit at McClean Hospital, spoke out early in the meeting, clearly distressed that a group at the quadrangle was going to usurp the name Neurobiology and possibly claim the field as its own. Even as the matter was brought to a vote, Jordi made one last ditch effort to

keep the name from the quadrangle group, but the Dean would not accept that. The neurologists and neurosurgeons also were divided in their support, with Derrick Denny-Brown and William (Bill) Sweet strongly in favor of the concept, while Ray Adams was opposed. Again, the opposition stemmed from concern that efforts to build clinically based research units would be jeopardized by forming a new department. After extended discussion, the matter was put to a vote and by a substantial majority, but not a unanimous vote, the Department of Neurobiology was formed.

## Political Activism

The 1960s were filled with serious, non-academic events of great magnitude. The Vietnam War, blatant racism in our universities, and the assassinations of Jack and Robert Kennedy and Martin Luther King, Jr. weighed heavily on us, raising our social consciousness, dominating our existence for periods of time during the decade, and making social activists out of all of us. They too are an important part of my life as a scientist.

### *A Program to Significantly Increase the Numbers of Minority Medical Students at HMS*

Three days after the April 4, 1968, assassination of the Reverend Dr. Martin Luther King, I received a phone call from Jonathan Beckwith, a colleague from the Microbiology Department at HMS. "We must do something about this at the medical school," said John. We agreed to convene a meeting the next evening in my house, with each of us inviting a few people who would be sympathetic to recruiting and training greater numbers of minority doctors. I invited my Neurobiology colleagues Ed Furshpan, Dave Potter, and Torsten Wiesel. John invited Luigi Gorini from microbiology, a fascinating man who had been a partisan in Italy during World War II, and who was responsible for saving thousands of Jewish youths from the death camps of the Nazis. Leon Eisenberg, Warren Gold, and Robert Buxbaum rounded out the group. That evening we drafted a proposal for the HMS faculty to substantially increase the number of minority students by establishing "fifteen suitably named scholarships per year" and by appointing a faculty committee to immediately implement the program. We wanted to name the scholarships after Reverend King. We recognized that with these proposals we were requesting a substantial change in the student population of HMS, which was predominantly white and male. In fact, HMS had averaged $\frac{3}{4}$ of a minority student per year in the 30 years prior to 1968.

The next morning we met with the Dean of the Medical School, Bob Ebert. He was sympathetic to our efforts and told us he would support us, but he also told us he could not do so publicly. We asked his advice on how to move this proposal along in order to have it approved at the next faculty

meeting, which was three weeks away. Ebert said that to have any chance of getting this approved, we would have to enlist the support of the heads of all the clinical and preclinical departments. We rushed back to the Neurobiology Department conference room to figure out how to proceed in this daunting task. We didn't know most of the people in the clinical departments, so how on earth were we going to convince them to support our efforts? Still, we plunged forward. First, we generated a list of the departmental heads and assigned members of our group the task of contacting them to arrange a meeting. We agreed that more than one of us would show up at each meeting and that these meetings would be wherever and whenever the Chairs were willing to meet with us.

We knew that we had to do much fact finding before the faculty meeting to head off what we anticipated would be partially hostile, but not necessarily unreasonable questions. Was there a large enough pool of outstanding minority students to fill 15 places in our medical school class? The answer to this question was easy. Yes, there was a large enough group of minority students out there, but Harvard would have to go beyond the small group of mostly Ivy League colleges that were its traditional sources of medical students. In fact, we were certain that special recruitment efforts would be required on our part to convince students attending urban or traditional black colleges and universities, where there were large numbers of minority students, that this was a sincere effort on our part. Would we be lowering our admission standards in accepting this large group of minority students? This question was harder to address. Part of the reason was that to HMS admissions committees, an "A" grade at Harvard carried much greater weight than an A grade at less elite institutions. In this climate, would the committee consider accepting credentials other than grade point averages and medical college aptitude tests for admission to HMS? For example, would the committee consider the running of a program for 50,000 youths in New York City (as done by one of the first students admitted in this program) a worthy criterion for admission to HMS? Would remedial training be necessary for these students and how would we arrange for that training? We actually anticipated that remedial training might be required for some students and suggested that it be made available on a voluntary basis by faculty. That suggestion, however, never was implemented because existing minority students considered it demeaning. Who was to cover the tuition and other expenses involved in bringing these students to HMS? Here, we planned to suggest the establishment of a Martin Luther King Scholarship Program to help cover the costs of bringing this new group of students to HMS.

### *A Contentious Faculty Meeting—April 26, 1968*

In the short time between our meeting with the Dean and the faculty meeting, we managed to gather the support of essentially all the departmental

Chairs. In addition, with help from a minority medical student, Noel Solomons, we identified, contacted, and gathered additional support from a group of key faculty whom he felt would be sympathetic to our cause. To present the petition to the faculty, we asked the help of some of the most highly respected members of the HMS faculty. We did this because we knew that if the petition came from a group of "radical" young faculty, we stood little chance of success with the conservative clinical faculty of that era. Elkan Blout introduced our resolution to add 15 minority students and to form a faculty committee to implement the program. Elkan was followed by Jon Beckwith, who, in explaining our selection of the number 15, also offered evidence that there should be little trouble finding qualified students from urban colleges and universities and via special programs that already existed, such as an "Intensive Summer Study Program" at Harvard and other leading universities. He also emphasized the importance of acting now. Members of the admissions committee also spoke up, including the highly respected Herman Blumgart, who documented the sad state of affairs then existing regarding minority enrollment at the medical school. Blumgart was concerned, however, about whether we would find suitable numbers of candidates. Other faculty also offered generally favorable remarks, but, then suddenly, things took a turn for the worse. Dr. Norman, a black Assistant Professor in one of the clinical departments, delivered what we sensed was a "we don't need your help, brother" speech. He commented that the Harvard admission standards were right where they should be and that they shouldn't be lowered to admit unqualified students of any ethnic group. He added that we would not have any difficulty finding qualified black students, but he was uncertain about the number 15. Then the floodgates opened, and many people spoke out against the number 15. Harold Amos tried in vain to stem the tide, but it was clear we were going to lose if we insisted on the number. After considerable rather chaotic discussion, the Dean asked Elkan Blout if the resolution could be modified to replace the number "fifteen," with "a substantial number." Elkan agreed and a vote carried the modified petition by a huge majority. Our group tried in vain to keep the discussion going regarding the number, since we felt that a substantial number might mean 3 rather than the $\frac{3}{4}$ of a student now in our medical classes. At that point, we actually were shouted off the floor by some of our clinical colleagues with cries of "sit down" and "shut up." The Dean, seeing the continuing confusion, called for a show of hands on including the number 15. Seeing that the faculty was seriously divided on the issue, he said that he would appoint a committee to look into the number. With that he ended the faculty meeting. The official minutes of the faculty meeting ended with the comment that we had made "a passage from the profane to the sacred during the course of the afternoon," since the first half of the meeting had been concerned with whether there should be a cap on clinical faculty salaries, leading to a huge turnout of the clinical faculty defending their rights not to have caps put on their salaries.

Our ad-hoc group gathered in the hallway outside the meeting room, furious about what had just transpired and feeling betrayed by the omission of the number. The Dean came over to us with a huge smile on his face and said, "What's wrong with you guys? Don't you know you've won? I said I would appoint a committee to look into the number and bring in a suggestion to the next faculty meeting, and *you* will be the committee!" Feeling somewhat sheepish, we quickly recognized that the Dean had successfully maneuvered our proposal through a reluctant faculty for what was to become an enormous and historic change in HMS and its student population. That change would implement what became and has remained the best program in the nation training minority physicians in a majority medical school. In the more than 30 years of existence of the program, close to 800 minority M.D.s have graduated from HMS, compared to about 25 in the previous 30 years.

*A War in Vietnam and "Strikes" on College Campuses*

With a notice sent by the deans of the Medical and Dental schools, an official day of mourning was announced "to mark the deaths of those students needlessly killed at Kent State University." The notice continued that "on Friday, May 8 (1970), the normal activities of Harvard Medical School will be suspended. All members of the medical community who do not have patient-care responsibilities are encouraged to devote that day to discussion and other constructive activities." This action was taken as part of a nationwide strike on college campuses to protest the latest horror of the most unpopular war in American history, the invasion of Cambodia, and in memory of the four students killed and nine wounded when the Ohio National Guard opened fire on unarmed students on the campus of Kent State University (May 4, 1970). It was the double horror of the expansion of the war and the invasion of our universities by the military that prompted the massive protests that followed.

My office was one of the Harvard Medical School Strike Centers, and I was the organizer of a teach-in that was scheduled for May 8. Our goal was to educate the clinical and basic science faculty and the student body about what was happening in Vietnam. With a small ad-hoc committee, and with very little time, we put together a program the likes of which had never been seen before on the Harvard Medical campus. We reserved and filled two amphitheaters for the event. Russell Johnson of the American Friends Service Committee described in graphic detail what actually was happening in Vietnam. Donna Howell of the Black Panther Party spoke next to explain what the Panthers were doing in the community that surrounded the medical school, including the running of a free medical clinic. Finally, Francis Moore, a highly respected neurosurgeon, who was not on the original program, requested and was granted time to talk about setting up a strike fund at the medical school. The formal lectures were followed by small group

seminars on topics such as the legality of the war, American imperialism, chemical and biological warfare, repression of the Panthers, and health care delivery in minority communities. The day ended with a roundtable discussion centered on what we as a medical community could do both to improve the delivery of health care in the neighboring Roxbury community and to end the Vietnam War. One outcome of that discussion led to a scene that probably startled many Bostonians and shocked some of the clinical faculty: Dean Bob Ebert, in his white coat, manned a table on a downtown Boston street giving out postcards to be mailed to the President of the United States supporting an end to the war.

I also organized a petition signing at the Medical School calling for the impaneling of a Federal Grand Jury to investigate the Kent State massacre in response to a phone call from the Kent State Student Council. Later, I organized a benefit showing of the film "Z" to benefit the families of students slain at Jackson State College in Mississippi, participated in the march of 100,000 people to the Boston Common to protest the war, and signed countless petitions to end the war. While this was going on, we kept the research going too, as we started moving in new directions.

## New Directions, Educational Enterprises, Special People (1970–1992)

### *New Directions: More Transmitters and Then Amines and the Modulation and Behavior*

**Glutamate as an excitatory transmitter compound at crustacean junctions.** Following our success in identifying GABA as an inhibitory transmitter compound, we turned to the question of whether glutamate was the excitatory transmitter compound at the same crustacean neuromuscular junctions. Physiological and pharmacological studies suggested that glutamate acted just like the excitatory transmitter compound, but we ran into serious problems in trying to demonstrate its release. The most difficult was the high background release of glutamate from neuromuscular preparations, requiring many experiments for us to see any release whatsoever. Still, we did see a selective liberation of glutamate with excitatory but not inhibitory nerve stimulation and in amounts comparable to those we had seen earlier in the GABA released by inhibitory nerve stimulation. Unfortunately, it took us 39 experiments to reach statistical significance, and that made it difficult to do controls such as attempting to demonstrate a calcium dependence of the release. Still, when we added in further elegant experiments from the Takeuchis in Japan showing that areas of high glutamate sensitivity on muscle fibers overlapped with excitatory nerve endings and that they too saw a small release of glutamate with excitatory nerve stimulation, most

investigators agreed that glutamate was the likely excitatory transmitter compound at crustacean junctions.

Thus, by the early 1970s, a definite transmitter role for GABA and a highly likely role for glutamate had been established using crustacean neuromuscular preparations. In vertebrate systems, by contrast, little progress had been made in demonstrating transmitter roles for these substances. Invertebrate preparations, however, had another huge advantage over vertebrate tissues in explorations of transmitter function. Lobster neurons were large, uniquely identifiable, and could be dissected as single cells free of contaminating neuronal tissues. Thus, in addition to clear-cut demonstrations of transmitter roles for proposed neurotransmitter candidates, single cell biochemical studies were possible that allowed us and other investigators to ask "just how different from each other were neurons using different substances as neurotransmitter compounds." Could transmitter function be changed by altering the levels of expression of key enzymes such as transmitter synthetic enzymes? Buoyed by our success with the amino acid transmitters, we asked whether we could identify other transmitter compounds in the lobster nervous system. If we could, would it be possible to explain transmitter accumulation in those neurons too by continuing the analysis of the levels of metabolic enzymes and substrates relating to that transmitter compound. Finally, by continuing that analysis would we be able to uncover general rules about how neurotransmitters accumulated in neurons? Would that give us any insight into the genomic regulation of transmitter accumulation in neurons?

**The "hot zap".** Before beginning our search for other transmitter compounds, we felt that a rapid method was needed to identify transmitter candidates. Thus, the affectionately named "hot zap" method was developed in the laboratory by myself, Dave Barker, John Hildebrand, and Ed Herbert (then on sabbatical with us in his first foray into neurobiology). In this method, we incubated tissue samples with high specific activity radioactive precursors of one or several of the known transmitter candidates. We followed the incubations by a single step, rapid separation of the precursors from products by high voltage electrophoresis (at 6000 V and 100 mamps of current—hence the name hot zap) and used the incorporation of radioactivity into a transmitter product to support a possible transmitter role in the tissue under examination. The method was sufficiently sensitive to detect synthesis of transmitter in single neurons. To illustrate the potential of the method, we used vertebrate sympathetic ganglia to demonstrate the synthesis of acetylcholine (ACh) and norepinephrine (leading to an elegant use of the method by Paul Patterson and his colleagues), small numbers of single leech Retzius cells to demonstrate a synthesis of serotonin (5HT), and lobster single cell bodies to demonstrate the synthesis of radioactive GABA in inhibitory neurons. We also tested the potential of the method to detect unknown transmitter candidates in tissues by using the full cocktail

of precursors in lobster nerve roots that either did or did not contain sensory fibers. Radioactive ACh was found only in the roots containing sensory fibers. We followed up these studies by showing that crustacean stretch receptor preparations, which contained single sensory neurons and their inhibitory innervation, synthesized only ACh and GABA. The hot zap was widely used by other investigators for a time, but soon became obsolete because of the need for special, rather dangerous equipment and because other methods of transmitter identification, such as the use of antibodies to localize transmitter synthetic enzymes, were becoming acceptable in the field to define transmitter function.

**Acetylcholine as the lobster sensory transmitter compound.** The preliminary studies with the hot zap were followed by more detailed studies on the possible role of ACh as the lobster sensory transmitter compound. Although it was well known that large amounts of ACh were found in crustacean and insect nervous systems, it was equally well known that in contrast to vertebrate systems, invertebrate neuromuscular preparations were insensitive to ACh and to agonists and antagonists that affected cholinergic transmission. Florey and colleagues had proposed that ACh might be the sensory transmitter compound in crustaceans after their bioassay procedure showed that little or no ACh-like material was found in excitatory and inhibitory motor axons, while large quantities were found in sensory nerve bundles. To examine this possibility in greater detail, we used an enzyme assay for choline acetyltransferase (the ACh biosynthetic enzyme) to measure levels of ACh synthesis in tissue extracts and the hot zap to demonstrate ACh synthesis by intact tissues throughout the lobster nervous system. First, we demonstrated a dramatic decrease in ACh synthesis in sensory nerve bundles in which nerve fibers had been severed from their cell bodies. Next, we examined different kinds of sensory receptors, showing in all cases that they synthesized ACh. In physiological studies we demonstrated that the cell bodies of central motoneurons were sensitive to iontophoretically applied ACh and that this effect was blocked by curare and atropine and potentiated by acetylcholinesterase inhibitors. Finally, we examined the physiological responses of an identified CNS motoneuron to stimulation of identified peripheral sensory receptors and showed that the resultant excitatory responses were blocked by cholinergic receptor antagonists. When Jim Townsel joined the laboratory, we carried out one final set of experiments involving sensory neurons. As with the excitatory and inhibitory motoneurons, we asked whether an analysis of the enzymes and substrates of ACh biosynthesis and degradation would explain the selective accumulation of ACh in sensory neurons. Here again, we found that the biosynthetic enzyme was found exclusively in sensory neurons and, therefore, was the key to accumulation of the transmitter product. The degradative enzyme acetylcholinesterase was uniformly distributed between all neuron types, being mainly localized in the sheath surrounding peripheral axons.

**Octopamine is the major amine synthesized from tyrosine in lobsters.** We turned next to the amine neurotransmitters, focusing first on amines derived from tyrosine because a candidate neuron already was available. Ian Cooke's laboratory had shown, using histofluorescence techniques, that a single large neuron present in the relatively small circumesophageal ganglion probably contained dopamine. This neuron sent processes to the plexus of neurosecretory endings in the pericardial organs surrounding the heart. Using standard biochemical procedures for isolating and measuring catecholamines, Dave Barker found anticipated low levels of dopamine, but could not detect any other catecholamines. In scouring the literature for other amines that might possibly derive from tyrosine, we found that in 1952 Erspamer had reported high levels of octopamine, the phenolamine analogue of norepinephrine, in the posterior salivary glands of the octopus. Could it be that, in invertebrates, the phenolamine octopamine replaced the catecholamine norepinephrine as the major amine derived from tyrosine? Perry Molinoff, a former student, was in Julie Axelrod's laboratory at NIMH at the time Dave began his studies. There Perry had just developed a highly sensitive and specific enzymic assay for octopamine, which he used to demonstrate that low endogenous levels of octopamine were found in vertebrate tissues. Perry in Axelrod's laboratory and Irv Kopin and his colleagues had earlier postulated that octopamine functioned as a "false transmitter" in the vertebrate nervous system, since it accumulated in sympathetic ganglia in large amounts after treatment with monoamine oxidase inhibitors and since it was released from these tissues with stimulation. Perry's results suggested that octopamine might serve a normal role in vertebrates as well, but with the very low levels of amine present it was difficult to determine what that role might be. When contacted, Perry jumped at the opportunity to see whether lobster tissues contained octopamine. To our delight, he found that octopamine was present and in much larger amounts in lobster nervous tissues than in any vertebrate tissue examined thus far. Thus began about a decade's worth of experiments exploring the role of octopamine in the lobster nervous system.

Bruce Wallace picked up the studies where Dave Barker had left off, starting with partially purifying and characterizing the lobster enzyme that synthesized octopamine from tyramine, the tyramine-β-hydroxylase. In all respects, the enzyme resembled the vertebrate dopamine-β-hydroxylase. Bruce also devised a highly sensitive assay for the enzyme that involved monitoring the release of tritiated water from side chain labeled tritiated tyramine. Joined now by Peter Evans and Barbara Talamo and using Perry's assay for octopamine and Bruce's enzyme assay, we started searching for the sites of highest octopamine concentration and synthesis in the lobster nervous system. To our surprise, this turned out to be along thin nerve roots associated with thoracic ganglia, which we had ignored in our first screens for the amine. These regions contained many orders of magnitude higher

concentrations of octopamine than any other place in the nervous system. Backfills of these roots with cobalt chloride demonstrated the presence of slender fusiform cells along the roots. Ann Stuart and Jim Hudspeth, who were in the department at that time, had accidentally discovered that the dye neutral red stained leech Retzius cells that contained serotonin. Thinking that this might be a general stain for amine neurons, we tried the dye on our roots and discovered the existence of about 120 of these cells in a bell-shaped distribution of numbers along all thoracic roots and the last several roots of the subesophageal ganglion. We were able to correlate the numbers of cells along a root with the content of and synthetic capability for octopamine. We showed further that octopamine could be released from the roots with depolarization by potassium, that dissected single cell bodies from the roots contained high levels of octopamine, and in physiological studies, that the cells were responsive to ACh. This set of results left us fairly certain that the root cells were octopamine neurons, and we suggested that these neurons, with their peripheral location, might be lobster homologues of the vertebrate sympathetic ganglia. It was not until almost eight years later, in studies carried out by Marge Livingstone and Sue Schaefer, that we fully realized how completely wrong we were.

For her thesis work with us, Marge had begun studies exploring the role of serotonin in the lobster nervous system. Marge noticed that not only were there high levels of octopamine along second thoracic roots, there also were high levels of serotonin at exactly the same locations. Working with Sue, who was an electron microscopist, they set out to localize the sites of serotonin and octopamine biosynthesis using combined electron microscopic autoradiography and biochemistry as their primary research tools. Their results demonstrated that four morphologically distinct categories of nerve terminals could be found close to the root cells, and of these, one was the site of serotonin synthesis and a second the site of octopamine synthesis. They found further that terminals of both these types surrounded the root cell bodies, making these cells different from all other lobster CNS neurons, in which no terminals were ever found close to cell bodies. These terminals would have contaminated the single cell samples examined, thereby misleading us to suggest that the cells themselves were octopaminergic. As soon as antibodies became available for octopamine (some developed by Barry Trimmer), the plexus of octopamine endings surrounding the cells were revealed in rich detail, and we recognized that the octopamine-containing cell bodies were found in central ganglia.

Almost 15 years later, Henning Schneider finally did map the octopamine neurons in lobsters when good antibodies became available, and even today, many of the roles served by these cells remain to be explored. While we were thinking that the root neurons were octopaminergic, however, Shiro Konishi and I carried out a detailed set of physiological studies on these interesting, but difficult to record from cells. We found that although

the cells were widely distributed along nerve roots in the nervous system, they shared synaptic input and were electrically coupled to each other, meaning that they might operate as a unit. Very recently, we found that the root cells contain one or more of the crustacean hyperglycemic hormone family of peptides, which are believed to be the lobster stress hormones. With an octopaminergic and serotonergic innervation, the root cells offer an excellent system in which to examine interactions between neurohormonal systems that are important in behavior at an identified cell level. A present graduate student, Alo Basu, is engaged in such studies now. Finally, with Mary Kennedy's help, we were able to show that lobsters do not metabolize amines via the vertebrate pathways involving monoamine oxidase and catechol-*O*-methyl transferase. Instead, amines are metabolized to single or double conjugates in which a sulfate group is added to the ring hydroxyl group and the amino acid β-alanine is added to the amino group. Such metabolites are expensive to synthesize, and we suspect that they will yet be found to have interesting physiological actions of their own, perhaps, for example, in signaling between organisms.

**Amines and modulation—we become neuroethologists.** Marge Livingstone was only a little way into her studies on the role of serotonin in lobsters when she made a remarkable discovery. We had been using neuromuscular junction preparations to examine the actions of amines, and later peptides, as modulators of synaptic function. Some early studies of these types were carried out by the Floreys shortly after serotonin was characterized by Rapport and his colleagues in the late 1940s. About a decade later, Grundfest and Reuben and Josef Dudel independently showed that serotonin increased the release of transmitter from excitatory nerve terminals in crustacean neuromuscular preparations. No connection was made, however, between the physiological actions that these investigators reported and a normal role for serotonin in crustaceans. The effects were being treated more as a pharmacological oddity than as a normal physiological mechanism. Our studies showed that serotonin, octopamine, and the peptide proctolin, which Tom Schwarz and Kathie Siwicki had recently characterized in lobsters, all had actions on neuromuscular preparations, but their sites and mechanisms of action varied. Thus, serotonin had presynaptic actions on excitatory and inhibitory nerve endings, while all three substances had postsynaptic actions on muscle fibers as well. Michael Goy showed that cyclic AMP could account for some, but not all of the actions of serotonin, while the actions of proctolin involved completely different mechanisms. Marge reasoned that if these substances were naturally occurring modulators as we were hypothesizing, and if they were not synthesized or released at muscle junctions, which we knew to be correct, then they were in fact hormones. Perhaps then, she would see something interesting by injecting amines into lobsters. I was certain that nothing of interest would result, as I expected amines to have actions at many sites in lobsters, and any consequences

of amine actions on so many targets would yield patterns of behavior too complex to interpret.

We were in Woods Hole during my tenure as Director of the Neurobiology Course when Marge rushed into the laboratory, took me by the hand, literally, and led me downstairs. There she showed me two lobsters, one standing tall, looking like a dominant animal, and the other standing in a lowered posture, looking like a subordinate. "What do you see?" she asked. I replied, "A dominant and subordinate lobster pair." "Wrong," she said and proceeded to explain what she had done. One of the animals, the one standing in the elevated posture, had been injected with serotonin, while the other, in the lowered stance, had received octopamine. These results immediately suggested an interesting possibility: perhaps as a consequence of animals interacting to establish a dominance relationship, serotonin–neuron function became more important in winners, while octopamine–neuron function became more important in losers. Subsequent to the interaction, longer term changes in the functioning of those neurons might reinforce the newly acquired behavioral patterns. Or putting it another way, social interactions might modify the function of amine neurons, and the modification of amine–neuron function might influence the outcome of future social interactions. That idea and exploring ways to test it have dominated our research interests to the present day.

**Serotonin neurons.** To ask whether changes in amine neuron function resulted from changes in social status, we devised the following research strategy: first, we had to find amine neurons in lobsters and learn to record from them; then we had to learn how they functioned; and finally, we had to ask if there were changes in function accompanying changes in social status. Our first task was to find the neurons. A talk in our department by Harvey Karten, in which he showed spectacular images of immunostaining for peptides in the vertebrate retina, prompted me to send Barb Beltz to Harvey to learn immunocytochemical methods. We knew that a good antibody was available from commercial sources for the detection of serotonin and anticipated that we would generate our own antibodies to octopamine. Using the commercially available antibody, Barb generated the first complete map of an invertebrate nervous system for serotonin. Her fluorescent images of serotonin immunostaining were so spectacular that we had trouble retrieving our original photographs from the editors of the *Journal of Neuroscience*, one of whom was using her figures as wall decorations. Barb's maps showed the existence of about 120 serotonin-immunostaining neurons in lobsters, which appeared to be organized in sets. Two pairs of these cells (one pair in the first abdominal, one in the fifth thoracic ganglion) were particularly prominent, sending processes from their ganglionic locations throughout the anterior part of the nervous system with ramifications of branches in every ganglion up to the subesophageal. These same cells send branches out all of the thoracic second roots that ended in varicosities close

to the root neurons described above, with second sets of varicosities seen in the pericardial organs surrounding the heart. Thus, these cells were capable of communicating with central neurons through their ascending branches and with all tissues of the body through their two sets of peripheral endings.

Meanwhile, Ron Harris-Warrick and Marge had shown that injected amines triggered opposite postural stances by directing the readout of opposing motor programs from the ventral nerve cord. Serotonin caused the readout of a "flexed" program, in which increases were seen in the rate of firing of excitatory motoneurons to postural flexors and inhibitory neurons to the extensors, while at the same time decreases were seen in the rate of firing of excitatory neurons to extensors and inhibitory neurons to flexors. Octopamine caused the readout of an "extended" posture by triggering opposite patterns of firing of the same groups of motoneurons. The readout of complex programs of these types from crustacean central nervous systems can be elicited by the firing of so-called "command neurons." Dominant animals assuming an elevated stance when in proximity to subordinates are seen in many species of animals. Therefore, we were not surprised to see this in lobsters too. In trying to identify amine neurons that might be important in fighting behavior, we began looking for cells that could exert central actions on the readout of motor patterns and also have peripheral actions on the muscles that were the targets of the motor readout. The large cells Barb had found seemed ideal candidates for the "right cells" to be working on, since their multiple sets of endings seemed able to reach both central and peripheral targets of the amine.

## Educational Enterprises: The MBL Neurobiology Course (1975–1979)

Along with the enormous growth of the field of Neurobiology through the 1960s and early 1970s, large numbers of investigators working on neuroscience research projects began filling summertime laboratories at the MBL. In 1954, 21 summer investigators identified themselves as neurobiologists; by 1970, the number had increased to 110. That represented 40% of the investigators in summer residence at the Institution. Major neuroscience discoveries had been made at the MBL, including Hartline's use of the eye of the horseshoe crab Limulus in the study of visual processing, and J. Z. Young's demonstration that the giant axon of the squid mantle was indeed a nerve fiber that could be used to investigate the mechanism of nerve conduction. Instruction in neurobiology began in the famous Physiology Course, whose origins dated back to the start of the MBL in 1888, and was continued in a highly successful Training Program in Neurobiology organized by Steve Kuffler, Dave Potter, and Ed Furshpan, which ran for 10 years (1957–1966). The Program included among its 74 "students" such luminaries as Seymour

Benzer, Larry Cohen, Don Pfaff, Denis Baylor, John Nichols, Jack Diamond, Pablo Rudomin, and Zach Hall. After a gap of several years, two new neurobiology courses were added to the MBL roster to replace the Training Program. One, an Excitable Membrane Biophysics and Physiology Training Program under the direction of Bill Adelman, began in 1969; the second, a Neurobiology Course under the leadership of John Dowling and Mike Bennett, began in 1970. During the summer of 1974, Jim Ebert, who was President of the MBL at the time, asked whether I would be interested in assuming the Directorship of the Neurobiology Course. He mentioned that the MBL would be discontinuing support for the Excitable Membrane Training Program the next year and wondered whether a new neurobiology course might cover some of the territory offered by that program as well. Basically, Ebert and the MBL Education Committee were dissatisfied with the educational value to the MBL of the Training Program and wanted it eliminated. I didn't realize the hornet's nest I was invading by taking on the latter challenge.

Before agreeing to become Director, I felt it important to line up a group of outstanding colleagues to help in the teaching of the course. First, I needed a Co-Director, and Tony Stretton was my first choice. Tony and I had rented a laboratory at the MBL that summer to search for dyes that could be used to optically monitor active neurons. That project grew mostly out of our looking for an excuse to work together again after the great fun we had in finding Procion Yellow a decade earlier. To my delight, Tony agreed, and together we generated a list of potential faculty to teach a course that would be divided into five blocks: Ed Furshpan and Dave Potter in neurophysiology; Tom Reese in neuroanatomy; Gerry Fischbach in cell culture; and Zach Hall (who then was back at Harvard) in receptor mechanisms. For the fifth block, Tony and I would add a biochemistry section. That group, we felt, would do an outstanding job of covering the area of cellular neurobiology. To our surprise, everyone was willing to teach with us, and with that we told Ebert we would give it a try. The Biophysics contingent was not happy with our choice as directors, let us know this, and withdrew funding for the new course from a private source of support they had acquired.

I have vivid memories of the evening before the first day of the course. It was Sunday, June 22, 1975. We were in a basement area in the Loeb building of the MBL, which was mostly a storage area and where part of the course was to be housed. A few rooms had been constructed in the basement for us by the MBL staff, one of which was designated as a course lecture room. None of the equipment in the laboratory was ready for the neurophysiology section, and we were convinced that there was no way we could get the course area ready in time for the students. A blackboard and screen had been placed in the lecture room, but the only illumination in the room was a

few old ceiling fluorescent fixtures that left the blackboard dark. We solved the board lighting problem by rushing to a hardware store in Falmouth and purchasing several clip-on lights which we wired across the ceiling of the room. That created a problem though, because when we plugged in the board lights we could not use the slide projector since there were too few electrical outlets in the room. Tables and chairs for the students had been scavenged from the old Mess Hall at the MBL and were in terrible shape. While sitting around moaning about the disaster that was about to befall us, Dave Potter began sanding one of the old oak Mess Hall tables. Slowly, we all joined in, grabbing steel wool and sand paper. One of us rushed to Falmouth again to purchase a clear lacquer to coat the tables. We spent the next several hours "finishing" the old tables, ending up making them look better than new. No one said "let's do the tables." Somehow or other, it just happened. After that completely unnecessary break, everything fell into place, and the course began, as scheduled, the next morning. New laboratories eventually were built for us in the basement in time for the third year of the course. The area was named the Grass Laboratory in honor of Ellen and Albert Grass for their loyal financial support of the course since its inception.

The course truly was a special experience for everyone involved. During each block, mornings were dedicated to teaching lectures, and afternoons and evenings, often running to 1:00 AM or later, were dedicated to the laboratories. Labs were always staffed with faculty willing to stay as long as students were there. Two research seminars a week, special symposia featuring invited guests (on topics such as Membrane Biophysics, Animal Behavior, Neuronal Peptides, and Vertebrate Central Nervous System), and special open-ended seminars on Saturdays where invited guests could talk at length on their field of study, all complemented the total immersion of the students in neuroscience. Evening parties capped off the special symposia with music, dancing, and margaritas, leading to long evenings of fun and relaxation for all involved with the course. Softball too was an important part of the course activities, with Coach Fischbach driving his charges hard in practice sessions before the games with other MBL courses. It was easy to spot the somewhat older group of Neurobiology students on the MBL campus. They were the ones with bandaged legs and arms resulting from muscles pulled during the softball games. The job of Course Director ranged from ensuring the smooth running of each block of the instruction program to making sure that paper cups were available for the morning coffee breaks.

There was considerable rotation of our faculty over my five-year tenure as Course Director. Tony Stretton, feeling the pressure of trying to run a laboratory thousands of miles away in Wisconsin while in residence at the MBL, finally had to drop out as Co-Director after the third year. My good friend and constant partner in wild new ventures, John Hildebrand,

succeeded Tony. John also shared the directorship of the course with Tom Reese for a second five-year cycle after I stepped down as Director. Paul O'Lague and his student, Sue Huttner, taught regularly in the neurophysiology section. Sue also was our course assistant for several years. In addition to being an excellent scientist, teaching in the neurophysiology block, and helping me organize all other blocks, Sue was the life of the course: she got the dancing going at all of the parties and made sure that students were out of the laboratory and on the field for softball practices and games. Gerry Fischbach's student, Ruth Siegel, also was a gem. She made sure the cell culture part of the course happened every summer, since, somehow or other, Gerry invariably had a hard time remembering things like ordering supplies for his section. Ruth taught in the cell culture section and also helped me generate an inventory of course supplies and equipment. Even more importantly though, Ruth kept me informed (to my amazement) about the complex social goings-on within the course every summer. John Heuser taught regularly in the anatomy section with Tom Reese, as did Philippa Claude, Story and Dennis Landis, and various students, postdocs, and former colleagues of Tom. Zach Hall taught for only one year and a receptor block was not reintroduced to the course until Jon Cohen joined our faculty in 1977. Finally, present and former postdoctoral associates and graduate students of mine and Tony's assisted in teaching the biochemistry part of the course.

The great joy of the summer was in teaching the exceptional students who took the course. With students like David Anderson, Mary Beth Hatten, Marge Livingstone, Jeff Corwin, Tim Ebner, Ben Peng, Jane Dodd, Jose Lemos, and Jose Garcia-Arraras, and more senior "students" like Hennig Stieve, Terry Sejnowski, Jerry Pine, Jerry Hurwitz, and Mike Zigmond, how could we miss? The pleasure of instructing this group was that they really wanted to know everything we could teach them, and they were insatiable in their quest for more. They were tired at the end of the summer, but felt invincible. There was nothing they could not do. Of course, reality set in when they returned to their home laboratories at the end of the summer. Still, all of us involved with the course felt a wonderful sense of accomplishment at the end of every summer. When my tenure as Director ended, the students threw a special party for me at which they unveiled a movie they had made over the entire summer, without my knowing anything about it. What was most embarrassing about my blissful ignorance was that son James was the cameraman for a script put together by David Anderson and Mark Noble. It was called "Abnormal Morphogenetic Movements" and featured, among other things, the famous nose/lobster claw transplant experiments. At the end of these five intensive years, I was at a bit of loss about what to do next, besides research that is, to enrich my life. I didn't have to wait long though as The Neurobiology of Disease Teaching Workshops, joining the Hereditary Disease (HD) Foundation Board, starting Neuroscience Commentaries, and

beginning the Harvard Program in Neuroscience soon filled whatever void I might have been feeling.

## Special People: The Wexlers, Marjorie Guthrie, The HD Foundation, and The Neurobiology of Disease

*First Meeting*

I first met Nancy Wexler and Marjorie Guthrie (second wife of Woody Guthrie and mother of Arlo) at an NINCDS-sponsored Long-Term Strategies Planning Panel on Inflammatory, Demyelinating and Degenerative Diseases, held in Williamsburg, VA, in May 1978. Nancy, a Health Scientist Administrator at NINCDS, was an observer at the meeting, and Marjorie, the President of the New York-based Committee to Combat Huntington's Disease, was there to speak about Huntington's Disease. Nancy, Marjorie, and Milton Wexler (Nancy's dad) had in 1977 worked together as members of a congressionally mandated commission for the Control of Huntington's Disease: Marjorie was the Chairperson, Milton was the Vice Chairperson, and Nancy was the Executive Director of the Commission. As an observer at the Planning Panel, Nancy was supposed to sit quietly in the background and take notes. Being Nancy, of course, she'd have none of that and was outspoken on many issues relating to disease-related science, contributing intelligent, thoughtful, and provocative comments to much of the discussion that followed the formal presentations. Marjorie described the commission report and delivered a stirring presentation on the role of Health Voluntary organizations in the battle against degenerative diseases.

Without doubt, Nancy and Marjorie were the most lively participants at the panel meeting. They spoke about neurological diseases with such passion that I went out of my way to meet both of them and to invite them to speak at the MBL Neurobiology Course that summer. It was their presentations at the panel meeting that made me recognize that we never mentioned neurological diseases and disorders in our course. As far as our students were concerned, and as far as we were teaching them, the nervous system always functioned properly. That summer and the next, Nancy and Marjorie visited and lectured in the course. Their inspiring presentations excited the students and kindled in me the desire to do something about trying to interest next generation scientists in the poorly understood and mostly untreatable neurological and psychiatric diseases and disorders that afflicted countless millions of people throughout the world. It was over lunch at the Fishmonger Restaurant in Woods Hole during Nancy's second visit to the MBL in 1979 that five of us, Nancy, Alan Pearlman, Michael Zigmond, Dennis Landis, and myself, formulated the outline for a national course to

teach young people about disease (The Neurobiology of Disease Workshops and the Harvard course that followed).

*The Hereditary Disease (HD) Foundation*

My ties to Nancy, Alice (Nancy's sister), and Milton Wexler and their Foundation began when Allan Tobin, who was the Scientific Director of the Foundation, invited me to attend an International HD Meeting and a Foundation workshop at the Hotel del Coronado on Coronado Island near San Diego in October 1978. The invitation likely derived from our earlier work on GABA neurons, which are the first to die in the brains of patients with HD, and from my interactions with Nancy at the MBL course. A letter to Milton summarizing my impressions about the research presented at the meeting and workshop ended with "I hope I can assist you in other ways in the future. One cannot help but become involved when one is confronted with people like you and Nancy (in particular), Jennifer Jones Selznick and your crew of young enthusiasts and your devotion to this cause." Allan invited me to join the Scientific Advisory Board of the HD Foundation in December 1979, and I was elected to the Board in January 1980.

When I first joined the Board I was impressed with the dedication and enthusiasm of the members, most of who had been with the Foundation from its inception. I was disappointed though at the quality of much of the research they were supporting. Part of the problem was that grants were going to Board members, some of whom were not doing forefront research, but a more important part dealt with the quality of the applications the Foundation was receiving. At one of the first meetings I went to, there were two applications investigating whether membrane defects existed in fibroblasts in patients with HD. Both of these laboratories were supported by the Foundation, and they continually reported opposite results in their studies. Instead of continuing to support both groups, I suggested that a single grant be given to the investigators involved, with the requirement that they work together on the project. Unfortunately, or perhaps fortunately, that put an end to Foundation support for those studies, since neither of the principal investigators was willing to meet the required condition. In March of 1980, before my official Board duties had begun, I organized a workshop for the Foundation on "Cell Death." I was surprised that this was not a dominant theme in earlier workshops. As I saw it, two striking facts defined HD, and these I believed should become the focus of the Foundation's research efforts: one was that HD was an autosomal dominant disorder, and hence, a search for the gene should be undertaken; the other was that neurons died in the brains of Huntington's patients, and hence, understanding how, why, and where neurons died should become a central research theme.

If I had to evaluate my time on the Board, I would say I played a role in several major changes in the directions of the Foundation. One was in

my very strong support for David Housman's initiative to use restriction fragment length polymorphisms (RFLPs) to try to find the mutated gene. Here is what I said about this in a letter to Milton Wexler reporting on the January 1980 workshop. "What seemed to me by far the most useful and far-reaching technology in relation to disease that we heard about, however, was the work David Housman described on the attempted cloning of human genes. There seems to be little doubt that this will work. All of the techniques that are needed now are available to the molecular biologists and while I am certain that problems will arise ... this seems to be a most promising avenue to a pre-natal diagnosis of human disease. I agree entirely with Bill Dreyer (who was on the Board) that within the decade this will be done (and should be strongly supported financially), but I don't agree that we will understand all about genetic diseases of the nervous system. One still has to know what the mutant gene is producing and where it fits into an animal's behavior to produce the disease." In a way, my comments were prophetic. Everyone was very surprised at how quickly a linkage to HD was found by Jim Gusella (who had started out with David Housman on the project) in 1983, leading to the isolation of the mutant gene Huntingtin 10 years later. But, 22 years later, one still does not know how the gene functions in producing the disease. With the development of excellent animal models of HD and other degenerative diseases of the nervous system, however, investigators are coming closer and closer to understanding the disease process. Hopefully, this also will lead to new therapies.

Another important role I played on the Foundation Board was in pushing to move in the direction of understanding the mechanism of neuronal cell death. I felt even if one did not succeed in the genetic approach to understanding HD, if you knew how neurons died and if there was a final common pathway of neuronal cell death, it might be possible to slow the death process and thereby reduce the ravages of the disease. I introduced Bob Horvitz to the Foundation at the Cell Death Workshop I organized, and he soon joined the Board and became one of its strongest and wisest supporters over the years. Bob has just shared the Nobel Prize for Physiology and Medicine for his and Junying Yuan's original work in *Caenorhabditis elegans* on cell death.

As one of my last contributions, Steve Matthysse and I originated the concept of "Collaborative Research Agreements," which invited outstanding researchers to work in HD-related research by offering them partial funding for directed studies. Unfortunately, our first venture in these directions was not well treated by the Board. As the first candidate for this award, we selected an HMS researcher who had developed excellent new methods to generate monoclonal antibodies that were highly selective for nervous tissue of different types. Steve and I felt that this investigator actually might be able to generate specific antibodies to HD-diseased tissue, and hence, our support for the project. Unfortunately, we were accused of nepotism for

having selected an HMS investigator. We still felt the Collaborative Research Agreements were a good idea, and ultimately, a more mature form of these agreements was awarded to a collaborative that included some of the best research groups in the world working on human disease-related genes. That was, in fact, the group that isolated the mutant Huntington's disease gene.

Although my membership on the HD Foundation Scientific Advisory Board lasted only 4 years (I stepped down, deciding not to serve for a second term), this was a most fulfilling adventure for me, opening new horizons and new dimensions in my life. It also allowed me to form long-lasting close friendships with the amazing Wexlers, Milton, Nancy, and Alice and to interact with their dazzling array of celebrity friends.

## The Neurobiology of Disease

### *A National Course—The Neurobiology of Disease Teaching Workshop*

After Nancy's second visit to the MBL in 1979, we formulated a plan for a national workshop whose goal would be educating young scientists about the diseases and disorders of the nervous system. The plan incorporated what I believed were the best elements of the HD Foundation workshops, the ways that Ed Furspan and Dave Potter taught medical students, and the way I taught graduate courses at Harvard and at the MBL. Even today, I feel that this is the most creative educational enterprise I ever have been involved in.

To attract an audience for these national workshops, we felt it important to link them to the Annual Society for Neuroscience Meeting. Therefore, during the fall of 1979, Nancy and I met with Sol Snyder, who was President of the Society for Neuroscience at the time, to get approval for this affiliation. Sol liked the idea a lot, gave us a go ahead to carry out a first workshop on a trial basis at the Society Meeting in 1980, promised us administrative support from the Society, but said that the Society would not offer financial support for the enterprise. Thus, the bottom line once again was that we would have to raise all the money required for the workshop ourselves from foundations or federal sources. This was not an easy task. Even the HD Foundation was not enthusiastic about the idea at first, awarding us only a small sum of money that covered only a fraction of the anticipated expenses. The Head of the National Multiple Sclerosis Foundation (M.S. was one of the diseases we planned to cover the first year) wondered how we could possibly cover their disease in one 3-hr session. He patiently listened to my description of our concept and then carefully explained how they organized meetings that went for many days covering only small areas relating to their disease. I felt like saying, but didn't since we hoped to get some money from them, that I didn't really need 3 hr to teach students all that was *really* known about M.S. I could do that in 30 min. The M.S. Foundation

gave us nothing the first year. Somehow or other, I did piece together the needed funds, going to friends such as the Bay Foundation (Bob Ashton), Merck Sharp and Dohme (Roy Vagelos), and the Klingenstein Fund. Even NINCDS officials like Katherine (Kit) Bick, who really liked the concept of the workshops, did not support us until the third year of the workshops.

Our plan for the workshops was that they would last two days, and we would cover different diseases on each day. Both days would begin with a patient presentation followed by core clinical and basic science lectures. Nancy, Allan Pearlman, and I traveled the country searching for outstanding teachers to deliver the core lectures. We emphasized that we wanted real teaching lectures and not research seminars. Our aim was to build a base of knowledge for our students about where research was in the field and where it might go in the future. Even more, we wanted students to begin to think about how their own research might fit in—how it might be relevant. To ensure that the core lectures really would be cores of knowledge, we required that faculty delivering these lectures "practice" and refine them at a premeeting held several weeks before the actual workshop. The audience for these rehearsals would be the organizing committee and other faculty participating in the workshop. We figured that if we as a group could not understand the lectures, there would be no way that students unfamiliar with the topic would understand them. These turned out to be amazing, fun, and intellectually satisfying sessions with a distinguished faculty from all over the country arguing about what the core facts were and how best to present them. Some faculty even admitted that after these sessions they delivered the best lectures they ever had presented. At the workshops, core lectures were followed by small group discussions in which students were encouraged to talk about what they had just heard and to speculate on how research might "solve" these difficult, intellectually challenging problems. Each day ended with special workshops in which investigators expert in new technologies would brainstorm with students about how their technology might be applied in the battle to conquer disease. In the evening, a banquet was held featuring a speaker from a health voluntary organization talking about the human side of disease—how these tragic diseases impacted of families. The workshop ended on the second day with a presentation by Nancy about how to apply for funding from the NIH. Our goals were to totally immerse students in these diseases for the two days of the workshop and, of course, to hope that some of them actually would begin to work in these areas as well.

The first workshop was held at The University of Cincinnati Medical Center under the sponsorship of their Neurology Department on November 8 and 9, 1980. The themes were autoimmune diseases—myasthenia gravis and multiple sclerosis—on the first day and degenerative disorders—Parkinson's and Huntington's Diseases—on the second day. Jon Lindstrom delivered our lead-off talk with his research showing that myasthenia gravis

was an autoimmune disease in which patients developed antibodies to their own ACh receptors. We felt that Jon's work was the model of what the workshops were all about—how basic science could make a fundamental contribution to an understanding of a neurological disease. Marjorie Guthrie gave the evening lecture on her "Personal Experiences with Huntington's Disease." One student gave Marjorie's talk a rating of 10,000 on a scale of 1–10. Another student commented that he "would steal hubcaps" to attend the next year's meeting. I have vivid memories of Nancy and I running around the halls of the medical school at the end of the workshop locking the doors of rooms used during the small group discussions, thoroughly thrilled that we actually had pulled it off: the workshop had happened and had been an enormous success. Twenty-three years later, the Neurobiology of Disease Workshops still are going strong. They now are reduced to a single day before the Society Annual Meeting, but still have NINCDS support and present the ever-growing base of scientific information to enthusiastic audiences of young investigators interested in going out and doing something to effect a cure for these diseases.

*The Neurobiology of Disease Course at Harvard*

With the national course off to a terrific start, I felt it important to have a Harvard version of the course too. Joe Martin agreed to be Co-Director and we offered the first cycle of the course during the fall semester of 1983. Joe and I generated a list of diseases and disorders that we planned to cover over the semester and selected faculty teaching teams who we felt were good teachers and experts in the clinical or basic science aspects of each topic. Occasionally, we found one investigator who could do both. In designing the course we kept as many of the features of the workshops as possible. Thus, we included patient presentations, teaching lectures as opposed to research seminars, and a survey of the current literature in the field. The emphasis was to be on what was and what was not known about the disease and how basic science might contribute to understanding the disease and help in the development of new therapies. Each week we presented a major disease or disorder, had patient presentations and core clinical and basic science lectures, had student presentations of current literature, and extended free-form discussions of the topic. Topics the first year included myasthenia gravis; the muscular dystrophies; Alzheimer's, Huntington's, and Parkinson's diseases; affective disorders; pain; and others.

As with the national disease workshops, I offered to rehearse faculty prior to their presentations, but none of my Harvard colleagues took me up on the offer. Still, faculty really did enjoy their involvement with the course. Marcel Mesulam wrote: "I have taught in more courses than I care to count. However, I would like you to know that the two sessions in your course on the Neurobiology of Disease have been just about the most enjoyable

teaching experiences I have had." Gerry Klerman added, "This experience convinced me that we need to do more teaching about the pathophysiology and neurobiology of psychiatric disorders." The students gave us the highest ratings of all the graduate courses offered at the Medical School. Faculty and former students who participated in the early years of the course have come back year after year to teach with us. This has been particularly rewarding for me because we ask a lot of our faculty and do not offer any recompense for their efforts.

Actually, it was hard to miss having an outstanding course when the first students included Ben (then Barbara) Barres, Peggy Mason, Junying Yuan, and Tony Monaco, all of whom have gone on to distinguished research careers working at the clinical/basic science interface. Tony's thesis was concerned with cloning the defective gene for muscular dystrophy. He was in his first year of study, when Lou Kunkel, teaching in the disease course, outlined a strategy for cloning the defective gene in Muscular Dystrophy. Excited by the lecture, Tony went to see Lou the next morning and said he wanted to work on the project. Early the next week, the work began. The result is history. That outcome represented much of what the Program in Neuroscience and the Neurobiology of Disease National Workshops and Harvard course were all about for me. Finally, we were starting to see progress in understanding the diseases that so touched me as a camp counselor so many years ago. Possibly, just possibly, I had played some role in facilitating that progress.

## Neuroscience Commentaries—A short Lifetime for an Exciting Adventure (1981–1984)

When Eric Kandel, the President of the Society for Neuroscience, first asked me to edit a new section for the Neuroscience Newsletter, I turned him down. Eric felt that the newsletter was boring, containing almost exclusively Society business, job postings, and meeting announcements. He recognized the great excitement that neuroscience research was generating, that the field was growing at an enormous rate, and that the Society should be doing a better job of informing the membership of what was happening in the field. The model that Eric had in mind was a "News and Views" section like the one that existed in the journal *Nature*. Even though I said no, I was intrigued by the concept of playing a central role in keeping the membership of the Society informed about current trends in neuroscience. Later, at the same Society meeting, in brainstorming sessions with two of my favorite people, Nancy Wexler and John Hildebrand, we began discussing the kind of vehicle that might be launched to explain the field in an exciting and useful way. We felt it important to not only reach the membership of the Society with whatever we offered, but to go farther—to the press, the public, and Congress, who, after all, were going to fund the science and should know what we, as a

field, were doing. With my and Nancy's heavy involvement in disease-related issues at the time, we also felt it important to aim whatever basic science we presented toward the solution of clinical problems. Together, Nancy, John, and I formulated and presented Eric with the idea of a new mini-journal called "Neuroscience Commentaries." He immediately liked the concept and encouraged us to assemble a first issue to be enclosed within the *Neuroscience Newsletter* and sent gratis to the membership of the Society. Eric already had acquired a $5000 grant from the Klingenstein Foundation (and our good friend Bob Ebert) to begin the venture, and together, he and I acquired a second grant of $10,000 from the same foundation to continue publication after the first issue came out. John, Nancy, and I agreed to serve as Interim Editors to get *Commentaries* off the ground.

Our plan was to publish three or four issues of *Neuroscience Commentaries* a year that would include "clusters of essays, loosely organized around a common theme, that would consider the historical perspective, the methodology, and the recent advances of key research groups in the thematic area; brief summaries of these essays aimed at nonscientists; articles examining issues arising at the interface between the neuroscience community, the government and the press; and reports of services available to neuroscientists such as brain banks, cell culture facilities, and sources of research materials, including antibodies, enzymes, drugs, and experimental animals" (from an editorial appearing with the first issue). The first lay translation was included with the second issue and was brilliantly done by Julie Miller, who had received a Ph.D. from the Harvard Department of Neurobiology some years before and who later went on to found her own journal, *Bioscience*. We also received outstanding editorial help from Gerry Gurvitch in the offices of the Society for Neuroscience, who with great skill, good humor, incredible patience, and occasional poems kept us on target in getting issues of *Commentaries* published and sent out to the membership. Gerry was our Managing Editor. All of the work involved in getting *Commentaries* off the ground was done without remuneration to John, Nancy, or myself. One difference between *Commentaries* and other review journals of the day was that the three of us did extensive rewrites of the articles, something our authors were not used to. The quality of the final product, however, was well worth it, as all the articles ended up eminently readable. Eric remained our strongest supporter, continually applauding our efforts, trying through all his varied connections to get us funding for the journal, and arguing with the powers that be within the Society for allowing us to maintain the free-ranging and unfettered style with which we were operating.

Three issues of *Commentaries* appeared before its demise: the first, in September 1981, was on "Peptides in the Nervous System"; the second, a year later, was on "Neuronal Cell Death"; and the final issue, published in December 1983, focused on the ACh receptor as the prototype for the mediation of fast synaptic responses. A survey of the membership showed

an overwhelmingly favorable response to *Commentaries* from the 500 or so members who responded. In addition to Eric's support, the Chair of the Publications Committee, Sam Barondes, and the Editor of the *Journal of Neuroscience*, Max Cowan, also were full of praise for our enterprise. So what killed *Neuroscience Commentaries*? Actually, it was a combination of several factors. Despite multiple applications, including one to NIMH, neither Eric nor I could get any long-term funding for *Commentaries*. We did receive a $20,000 grant from the Sloan Foundation that required matching support, but none was forthcoming from the Council of the Society for Neuroscience or from any other sources. We also felt that many members of the Council were lukewarm in their support for *Commentaries* and didn't see anything unique in what we were trying to do. One suggestion was to fold *Commentaries* into the *Journal of Neuroscience*, but we felt that Max Cowan was not very enthusiastic about that possibility. Max liked *Commentaries*, but did not want an independent venture as part of "his" journal. Finally, after much discussion, a complete submersion of *Commentaries* within the journal was the option offered us by Gerry Fischbach, who was then President of the Society. The terms outlined for this were not satisfactory to any of us involved with *Commentaries*. The three of us resigned, hoping that the Society for Neuroscience would try to keep the concept alive, but along with us and the three issues that were published, *Neuroscience Commentaries* disappeared into the proverbial sunset.

## Colon Cancer (1982)

*I wish I had the voice of Homer*
*To sing of rectal carcinoma,*
*Which kills a lot more chaps, in fact,*
*Than were bumped off when Troy was sacked.*

*So now I am like two-faced Janus*
*The only god who sees his anus.*
*I'll swear, without the risk of perjury,*
*It was a snappy bit of surgery.*

Excerpts from "Cancer's a Funny Thing," a poem by J.B.S. Haldane

Someplace in the middle of all of this, I was diagnosed with colon cancer. Therefore, this section is placed someplace in the middle of all the other sections covering this period of my life. The statistics on survival with colon cancer were that 50% of people with the disease would die within one year of diagnosis. I decided that I would be in the other 50%. I really wasn't ready to die, and that was that! There were many things that had to be done in a very short period of time though, just in case things didn't work out the way

I expected. First and foremost, my family had to be protected. Kathryn and I had not drawn up a will, which therefore had to be done immediately. Then, who was I to tell about the upcoming surgery? My family had to know, the lab group had to know, and a few special friends had to know. I decided that was as far as I would go. I also felt it was essential for someone to be in the operating room during the surgery to take a piece of my tumor. This too was just in case the prognosis for the future was not as rosy as I anticipated. My plans were to make monoclonal antibodies to my tumor, attach a toxin to the antibodies, and inject them into myself to try to destroy the tumor. After all, I was a scientist, and I was not going to die without battling every inch of the way with whatever special skills I could muster as a scientist. I also had read that human tumors could be placed in cell culture in order to devise a rational strategy for chemotherapy. For this too, I needed a fragment of my tumor.

A few days before surgery, the members of the lab group asked to meet with me. Tom Schwarz said, "Ed, we can't understand how you can be so calm about this." My response was, "I have an awful lot to do in a very short period of time to protect my family, myself and all of you, and if it helped to be hysterical, you can be sure that I would be hysterical." Michael Goy generously agreed to continue teaching my course for me, and with everything as well in hand as possible, I went off to have my colon removed. Part of the anesthetic involved an injection of morphine into my spinal cord, and the resident who was doing the injection was a former medical student of ours. She remembered me, was a bit nervous, and then missed getting into the spinal cord on the first try. I was thinking at the time that I wish she didn't remember me. With help from Art Pardee, Dr. Howard Fingert was in the operating room to take a fragment of my tumor for research purposes. Luckily, it wasn't needed. When I awoke from anesthesia in the recovery room, John Brooks, my surgeon, was standing over me. He said, "We have a cure!" What a relief those words were. The first two people to visit me when I awoke in my hospital room were Dan Tosteson, who was the Dean at the time, and Nelson Kiang, a colleague and friend. Both came in to see me with big smiles on their faces and with books for me to read. I have no idea how they found out about my surgery.

## The Program in Neuroscience (1982–1990)

In 1981, a university-wide graduate Program in Neuroscience was established by the Harvard University Committee of Biological Sciences. I was offered, and accepted, the Directorship of this Program. With the enormous growth of the neurosciences in the nation, and with more and more hiring of neuroscientists by HMS, Harvard College, School of Public Health, and Harvard-affiliated hospital departments, the time was ripe to form a broadly based new program. The goals of the Program, as I viewed them, were (1) to bring together basic science and clinical faculty engaged in neuroscience

research throughout the University and (2) to attract the best graduate students in the nation to this emerging and rapidly growing field of study. I managed to negotiate a budget from the Medical School for a student office, a Program Coordinator, and an Associate Director to run the Program, which may have been the first time that a cross-university program of this sort had its own budget. Before accepting our first students though, I had to build a faculty and establish an academic teaching program. To do this, I had to convince investigators engaged in neuroscience research throughout the University that we were going to make a serious effort to build a focused research community out of what was a widely scattered assortment of investigators. One reason for skepticism about the seriousness of my intent was that the Neurobiology Department did not allow faculty appointments outside the physical confines of our quadrangle-based department. This policy had earned the department the reputation of being elitist, but the policy of exclusion had its purpose. Steve desired to maintain a strong, coherent, manageably sized unit that could be run more like a family (as was Steve's style) than like the corporate entities typical of other medical school departments of the day.

Personal visits to other centers of concentration of neuroscientists, permission from the Medical School to make appointments in the Program in Neuroscience (another first), a firm commitment from me that graduate students would be shared among all faculty, and a guarantee that affiliates would play roles in the planning and running of the Program soon led to applications by many faculty members to join us and wide representation of many departments throughout Harvard and its affiliated hospitals in the Program in Neuroscience. In meeting my promise of faculty involvement, I established two administrative committees that ran the Program: an "Executive Committee" of senior neuroscientists and administrators that was responsible for overview of the Program and approval of faculty affiliate appointments and a "Working Committee" of younger faculty that actually ran the Program. Quite early in these efforts, I had the good fortune to have Tom Fox join me as Associate Director.

From the start to the finish of my tenure as Director (1982–1990), the Program in Neuroscience was student oriented. We did attract the truly outstanding students we were looking for. Included in our early classes were Ben Barres, Tony Monaco, Peggy Mason, and Junying Yuan (who started out in Neurobiology, but transferred to the Program after 1 year), among others. Starting with five accepted students in 1982, the Program grew by 1988 to 34 students (15 Ph.D., 10 M.D./Ph.D., and 9 M.D. returning for their Ph.D.) and 90 affiliated faculty representing 12 different research centers. Tom and I felt strongly that the center of the Program in Neuroscience was our students. Therefore, we did all we could to enrich the quality of their lives. Excellence in scientific training and research was stressed, of course, but an intimate involvement of the students in running the Program and

in having fun were not forgotten. Potluck dinners, open office hours, two annual retreats on Cape Cod (one for students only, the other for faculty and students for symposia in which the students selected and hosted the speakers), and a revival of BANG (the Boston Area Neuroscience Group) all were for our students. We involved them in organizing and running as many of these events as possible. Tom and I usually were invited to the student's Fall Retreat, but we suspected that was mainly to make margaritas and start the dancing. The two of us served a similar role in the much larger Spring Retreats (dancing and margaritas), which also featured occasional dips in chilly swimming pools by students and faculty who will remain nameless. Tom and I also began ethics discussion groups for our students, many years before they were an NIH requirement, to deal with issues that surfaced in interstudent relationships.

One nice benefit of the ability to grant titles to faculty outside the Department of Neurobiology was the good will that was generated between quadrangle- and hospital-based faculty. Very shortly after Seymour Kety was appointed Professor of Neuroscience in the Department of Psychiatry (February 1983), he wrote me a lovely note about the title stating, "I am very pleased with my new title and, in a meeting last week at which I was identified by that title, my colleagues at the conference seemed pleased as well." Clearly, the granting of titles identifying colleagues based in clinical settings as belonging to a quadrangle-based program was important in helping to build the community of scholars envisioned in the formation of programs of this sort by the University Committee of Biological Sciences. When Gerry Fischbach came to HMS to Chair the Department of Neurobiology in 1990, I stepped down as Director of the Program in Neuroscience, feeling that the Program was a success and that a new Chair should have a free hand to pick new leadership for the graduate program.

## Life as a Neuroethologist, Honors, Family (1992–Present)

*Life as a Neuroethologist*

**Serotonin neurons, quantifying behavior, and changes in neuronal function with changes in status.** In what follows, I present a few highlights from this decade of my life as a scientist. I limit my description here because I believe that the story of my accomplishments as a neuroethologist is in its infancy. The problem is that attempting to understand complex social behavior at the level of neuronal function is an enormous challenge, one that we still find ourselves learning how best to address. Mind you, I believe we have made interesting contributions to the scientific literature during this decade, but we still do not know how, or even if, serotonergic neurons

function during fighting behavior. Moreover, we have only the faintest outlines of notions about how a behavior such as aggression is assembled in nervous systems.

**The "gain-setter" role.** Barb Beltz had done a great job in finding the amine neurosecretory cells and in elaborating methods to routinely record from these neurons. She also carried out the first experiments that described the "gain-setter" role served by these neurons. Pokay Ma picked up on this theme and in a monster set of experiments elaborated the gain-setter story. His and Barb's results showed that serotonergic neurosecretory cells were part of the motor command circuitry, as expected, but in a much more interesting way than anticipated. First, the cells were not concerned with point-to-point wiring in the lobster nervous system. Stimulating cells through intracellular electrodes did not produce motor output from the ventral nerve cord, as had been seen with bath application of amines. Instead, when flexor command neurons were activated, in addition to turning on flexor motor programs, they increased the firing of serotonergic neurons, which in turn enhanced the output of the command. If extensor commands were activated, the serotonergic neurons were inhibited. If serotonergic neurons were forced to fire with an intracellular electrode when extensor commands were activated, however, the output of these commands too would be enhanced. Thus, the circuitry determined whether the serotonergic cells would show increased or decreased firing after activation of motor commands, and the serotonergic neurons would function as general gain-setters whose activation enhanced the output of motor circuitry.

**Autoinhibition.** It had been long known from studies in vertebrate systems that serotonin neurons showed autoinhibition, the property of turning themselves off after high-frequency stimulation. This was believed to be due to released serotonin having actions both on postsynaptic targets and on terminals that had released the amine to reduce further release. Therefore, we were not surprised when we found that after a period of high-frequency firing of A1-5HT neurons, there was a pause in the firing of the cells. Studies of this type were begun by Michael Horner, Don Edwards, and myself while at the MBL in Woods Hole the summer of 1997. They were elegantly continued by Ralf Heinrich at Harvard on our return from Woods Hole, with Stuart Cromarty joining in on some of the experiments.

There were two very interesting outcomes of these studies. One was that the autoinhibition seen in lobster serotonergic neurons did not result from the actions of released serotonin. What convinced us of this was (1) that the inhibition was seen in A1-5HT neurons from animals that had been treated with 5,7-dihydroxytryptamine, which depletes the A1-5HT cells of 5HT, and (2) that autoinhibition still was observed in the absence of extracellular calcium, which would prevent the release of the transmitter. Thus, "autoinhibition" appeared to be an endogenous property of these neurons: when forced to fire at high frequencies, they turned themselves off. In

the original description of autoinhibition by Aghajanian, such a possibility was discussed, but that notion disappeared from the literature. Possibly of greater interest, though, was that the duration of the autoinhibition was inversely related to the initial firing rate of the cells. Cells that fired spontaneously at low frequencies showed sustained periods of autoinhibition, while those that fired initially at higher rates showed little or no pause in their firing after high-frequency stimulation. Here was a surprising finding, for we had been paying little attention to whether A1-5HT cells fired at 1 or 2 or 3 Hz. Now we recognized that, within this narrow range, the initial firing rates of cells were important determinants of how the neurons functioned after high-frequency activation. Therefore, questions such as what set the firing rates of cells became issues we had to start thinking about. This might represent, in fact, a cellular mechanism whereby the way cells were used in the past influenced how they would be used in the future.

**A quantitative analysis of lobster fighting behavior.** I had been trying for years to interest a behaviorist in working with us to quantify lobster fighting behavior. I felt that this was important to do, because how else could we interpret pharmacological experiments we were planning to carry out involving changing amine levels in lobsters to search for effects on fighting behavior. If we didn't know what normal lobster fights looked like, how could we possibly interpret anything we saw with amine level manipulation. Jean Fraser, a behaviorist, worked with us for a while, and although she carried out some interesting learning experiments with lobsters, I couldn't convince her to analyze the behavior. We shared an NIH Program Project Grant with Jelle Atema for a while, and he too didn't seem particularly interested in performing the analysis (although a few years later members of his lab group did analyze fighting behavior in adult lobsters). Finally, on a trip to Texas Tech University to deliver a seminar, I met Robert Huber, who was finishing his graduate studies at the time. After my talk, Robert and I discussed the prospect of his coming to the laboratory to do the analysis that I had been so anxious to have done for so many years.

Robert was Konrad Lorenz's last student, and as such, he was a classically trained ethologist. However, he also was trained in evolutionary biology and in the use of computers for analyzing complex situations such as behavior. Thus, he appeared perfect for these studies. We thought it best to use young, socially naive animals that had never seen or fought with another lobster before to examine the elements that comprised the fundamental patterns of fighting behavior in lobsters. For this purpose, we used animals raised at the New England Aquarium that had not seen another lobster since the fourth larval stage when they began their benthic existence. The animals were between one and two years old (several inches in length), and to our great surprise, they knew all the rules of fighting behavior. Fights involved displays in which lobsters stood as tall as they could, showing their major weapons, the claws; limited aggression in which they held onto each

other with the claws and tried to turn each other over; and high-level aggression in which they grabbed onto each other with their claws and used short upward tail flips in an attempt to tear limbs off the opponent. Decisions could be made at any time during a fight, and once a decision was made, the behavior of both animals was changed. Recently, Rachel Rutishauser in my laboratory found that the changes in behavior can be detected as long as a week after initial decisions are made, demonstrating that long-term changes in behavior result from winning or losing fights. Robert showed further that lobster fights fit well with models of "game theory" in terms of their progression, the decision to retreat, and when they display and use their weapons.

One other line of investigation begun by Robert involved the pharmacological manipulation of amine levels in living, behaving lobsters. In the early 1980s, Marge Livingstone had shown that amines injected into lobsters triggered postural changes, but we hadn't followed up on her original studies to ask whether there were any other consequences of amine injections. Since a main theme in the laboratory was that serotonergic function might be enhanced in winning lobsters, Robert decided to inject serotonin into losing animals immediately after a fight to search for actions of the injected amine. He waited for the postural changes to decay away before pairing the now serotonin-injected losers with their former opponents. Once again, we got a surprise. The former losers now advanced on the winners, engaging them in fights, and fighting at intensity levels and for periods of time comparable to those seen at the start of the original fights. Robert got so excited at this result that he immediately paired the injected loser with a much larger animal, which promptly cut off the claws of the advancing smaller animal and killed it (the first and only time we saw a behavioral reversal in studies of these types). Our follow-up studies suggested that the observed effect was likely due to the uptake of the injected serotonin into serotonergic or other kinds of neurons and its subsequent release. We used Prozac-injections to determine that this was the likely scenario, which got us into Dave Berry's nationally syndicated column. We believed this behavioral reversal to be a motivational effect of the injected amine, although other possible explanations of the effect also were possible.

Robert's initial observations have been followed up by us, by him, and by other investigators using a variety of pharmacological reagents that raise or lower serotonin levels or effectiveness in lobsters and other crustacean species. The results of these studies, including our most recent ones, suggest that no simple relationship can be demonstrated between serotonin levels in crustaceans and agonistic behavior, at least not by using pharmacological manipulations that change amine levels or amine effectiveness in entire organisms. We still believe that serotonin is involved in aggression. It may be, however, that serotonin has to be released in appropriate amounts at the correct time, and in the correct place in the nervous system, to function

in complex patterns of behavior such as aggression. The relatively gross pharmacological procedures we have been using up to now may not offer the precision needed to influence behavior in a meaningful way.

**Fighting flies.** Lobsters seemed to be an excellent model system for studies on aggression. It was easy to get the animals to fight, and the patterns of behavior appeared to be prewired in the nervous system and modifiable by behavior. Anatomical and physiological studies could bring us to some of the neurons likely to serve important roles in the behavior and would allow us and other investigators to ask whether neuronal function changed as a consequence of winning or losing fights. Recently, our colleague Don Edwards showed changes in the serotonergic modulation of particular synaptic regions in crayfish as a consequence of changes in social status. Gene cloning and other kinds of molecular experiments also are possible with lobsters. So why stop working on a system that has yielded so much valuable information at this point in my career? The problem was that I felt we were at an impasse at ever getting closer to understanding how serotonergic neurons functioned in aggression or, in more general terms, at understanding how behaviors like aggression are assembled in nervous systems. Moreover, we were guessing at the neurons that were important in the behavior. It was an informed guess, of course, since serotonergic neurons appear to be important in aggression in all species of animals. But how would we ever discover new neurons or new pathways important in the behavior using a lobster model? The answers I felt might lie with an organism where the genetics already were well worked out, where the genome was available, and where a wealth of genetic methods were available for the asking—hence, the Fruit Fly Fight Club.

Sturtevant first reported that flies fight in 1915 in a paper on mating behavior in flies. There were more recent papers too, some dating to the 1980s, but even some of the world's greatest experts on flies, such as Seymour Benzer, didn't know that flies fight. The question was how to get them to do it in a simple enough experimental situation that the behavior could be quantified and that genetic approaches could be applied easily. Three excellent Harvard undergraduate students, Nina Bowens, Selby Chen, and Ann Lee, undertook the task of getting flies to fight in the laboratory. Our goal was to have just two males fight, so that eventually we might have a normal fly fighting versus a mutant to ask what effect the mutation would have on the behavior. As we learned more about the kinds of genetic methods that were available in flies, we realized that there were experiments we could do that were infinitely more elegant and more sophisticated than just making mutations.

We reasoned that flies would fight over the same sorts of resources that other animals fought over: territory, mates, and food. After much experimentation, we designed our fly fighting chamber which we affectionately called The Colosseum. It included glass walls made out of two standard

microscope slides that were cut in half and glued together at the ends, which were then placed on an agarose surface to supply humidity. A small 1-cm food cup was placed in the middle of the chamber, and a headless mated female was placed on the surface of the food. Mated so she was not as attractive to the males as a virgin female, and headless so she wouldn't fly off the food surface while the males were fighting. The males didn't care whether the female had a head or not. A petri dish with holes for ventilation served as the lid of the chamber, and a piece of dark filter paper restricted the light to the food surface. It was simple, but, more importantly, it worked. Within minutes of being placed in the chamber, males ended up on the food surface, and shortly after that, they began to fight. The fights were quite funny, including "wings-up" displays; fast and slow charges; pushing off with legs; grabbing; tussling; and my favorite, "boxing," where the two flies stand on their hind legs and duke it out. Of course, decisions are made in these fights, with winners and losers emerging as in other species of animals. To analyze the behavior, Selby and Ann carried out 75 fights, involving over 2000 meetings between the flies and more than 9000 behavioral transitions during those meeting. All these data were entered on computer spread sheets, and with Robert Huber's help again, we carried out a quantitative analysis of the behavior. With that in hand, the mutant studies now became possible.

These are in their infancy, and so will not be described in detail here. Our readings of the fly literature led us to the discovery of the powerful GAL4/UAS method originally described by Brand and Perrimon at Harvard Medical School about a decade ago. What this method will allow us to do when we have it fully operational in our laboratory is to essentially reach into the brain of a fly and reversibly turn on or off the function of any neuron types we are interested in, while the flies are fighting. It's like a dream behavioral experiment, and we are in the midst of carrying out these experiments as I write these words. We also will be able to ask whether changes in gene expression accompany changes in social status in flies, and using gene chips or other methods of analysis, we can quickly identify the genes that are changing and localize them in fly brains. Such studies will be carried out with Heinrich Reichert and Ronny Leemans of Basel, Switzerland, in the next few months. Hopefully, these lines of experimentation will allow us to get closer to the questions that have been driving my research efforts for the last two decades. Even if they don't, however, we are having fun thinking that they will.

### *Honors and Awards*

Honors are not why we do science. Still, recognition by peers is nice, and it certainly made my parents happy when they could read about "my son, the doctor" in announcements in New York newspapers. This article begins with a partial listing of my honors, so they will not be listed here. Comments on a

few of the honors might be worthwhile though. One of the more interesting happenings was that I almost turned down the invitation to become a member of the Institute of Medicine (IOM) because I knew nothing about the organization prior to 1986. I thought the letter was a ploy to get me to contribute money to a vanity organization. Luckily, I made inquiries and felt appropriately honored after I found out that they were medicine's equivalent of the NAS. Shortly after becoming a member, I served on the IOM Council (1991–1993) under two presidents, Sam Thier and Ken Shine. Shine was much more fun to serve under, as he was less of an autocrat than Thier. I don't remember that I was a particular success as a Council member though, as I had little practical experience working at the interface between medicine and politics. I suspect that I was there to represent the viewpoint of a basic scientist, since relatively few members of the IOM at the time were practicing scientists. Luckily, now large numbers of basic scientists are members who can be called to serve on the IOM Council. I am particularly proud of two awards on my list. One is a Lifetime Achievement in Mentoring Award, presented to me on December 2, 1998, as part of the A. Clifford Barger Excellence in Mentoring Awards ceremony at Harvard Medical School. This award comes via nomination from former students, and I am truly honored that my students went to this effort for me. The second is the Education Award from the Association of Neuroscience Departments and Programs that I shared with my colleagues Ed Furshpan and Dave Potter. That was presented at the Society for Neuroscience Meeting in San Diego on November 10, 2001. Since teaching has been such an important part of my life, I was greatly pleased to have this acknowledged by the organization representing neuroscience programs throughout the United States.

*Family*

I have already talked some about my wife of close to 45 years, Kathryn. She has been my constant companion, best friend, and the supporting and guiding hand in the raising of our two wonderful children. Along the way, she also has been a historian, a map maker, a social worker, and now a bible scholar. In fact, she has more degrees than anyone I know. Her latest, which took 14 years to complete from beginning to end, was a Ph.D. from Brandeis University in Near Eastern and Judaic studies on trophy taking in the ancient world. She therefore is a whiz in the bible category in Trivial Pursuit and is one of the very few people I've ever known who has taught Akkadian and who can read and understand Ugaritic, Aramaic, and ancient Hebrew. I've noticed that despite substantial differences in our upbringing, when we go to a synogogue for a wedding or bar mitzvah, I am reading the English translation or transliteration of the text, while Kathryn is reading, and understanding, the original Hebrew. Kathryn is extraordinarily well read, and somehow or other, even after 45 years together, we still find

much of interest to talk to each other about. That alone says a lot about our relationship.

Our son Dave was born February 21, 1964, and our son Jamie was born on May 14, 1966. Their childhood was, I expect, a fairly normal one, although we always felt it was special. The arts have been an important part of all of our lives, but more so in a professional way for the boys who make their living in these fields. Dave began singing and Jamie began making movies while both were in high school. Dave graduated from Swarthmore with majors in both music and science, while Jamie graduated from the honors program at the University of Michigan in film and video studies.

After college, Dave returned to the Boston area to attend the New England Conservatory in their opera program. Then he taught math, science, and Spanish for a while at the Commonwealth School, his high school alma mater. During that period, while singing for pay in a church choir, he met his wife to be, Majie Zeller. Majie, a lovely woman with a beautiful voice, also works as a project manager for the Lotus Corporation. Together, they moved to Ann Arbor where Dave got a law degree. This was followed by a clerkship in Boston with the soon-to-be Supreme Court Justice Breyer and then a second clerkship one year later with Justice Sandra Day O'Conner at the Supreme Court. All this high-profile law led to a position with a major law firm that left Dave no time for singing, a subsequent position as legal counsel with the Governor of Massachusetts, and now, a career doing legal writing at home so that he can have adequate time for his singing. Dave, a lyric baritone, and Majie, a mezzo soprano, fill our weekends with glorious music singing in Boston with the Cantata Singers, Emmanuel Music, and various local and regional opera companies. Of course, we wait anxiously with Dave and Majie to read the reviews of their performances and are delighted when reviews like this from "Opera News" show up: "The unequivocal show-stealer was baritone David Kravitz as Leporello. A natural crowd-pleaser, Kravitz sang with resonance and fluency, and he acted with an ease and expressiveness that far outshone the rest."

Jamie returned to the Boston area after leaving Ann Arbor and worked for the Cambridge Community Access Television station. He then moved to Los Angeles, where he set up the West Hollywood Community Access cable television station, initially teaching all their courses and beginning the station's regular schedule of cable casting. For a short while, he danced professionally with Naomi Goldberg's L.A. Modern Dance and Ballet troupe and the Rudy Perez Dance Theatre, culminating in performances in the 1993 Dance Kaleidoscope and L.A. Festival. His dance interests began in high school and continued at the University of Michigan, where he performed in and served as Co-Artistic Director of a modern/jazz dance group. Jamie met his partner, Sebastian (Bas) Uijtdehaage, an Assistant Professor at UCLA specializing in media and education, during this period. After stepping down as the Director of the community access channel, Jamie moved to a dot com

company for a while. More recently, while in transition between positions, Jamie made an award winning documentary called "Into the Streets" for the City of West Hollywood. This powerful, beautifully paced video documents the refusal in 1991 of Governor Pete Wilson of California to sign AB101, a moderate gay rights bill, and the stirring demonstrations that followed that refusal. It has been shown to acclaim at numerous film festivals.

I am delighted that both our sons are in the arts, that they are so intensely creative, and that they maintain the outspoken liberal beliefs that Kathryn and I so firmly hold to. The family was very proud to attend Kathryn's graduation ceremony, and Dave and Majie gave Kathryn a highly appropriate gift to celebrate the event afterwards at an elegant Cambridge restaurant. "Gave" is actually the wrong word. Since her thesis was on "trophy taking," she was shown one of those large cups given to athletes who win national championships, which she then had to wrestle away (take the trophy) from Dave and Majie in the restaurant. In a way that tells a lot about my wonderful family. We are, and always have been, very close, warm, and affectionate toward each other. We maintain a sense of humor and a sense of perspective in and about everything we do. We take pride in each others accomplishments and share in their celebration. We honor, respect, and support each other. Who can ask for anything more!

## Epilogue

Steve Kuffler used to say "the good old days are now." He meant that in the best sense, which was don't look back with nostalgia at what used to be. It's a philosophy I agree with, and this autobiography, therefore, is not an attempt to offer a sentimental view of my good old days. The first decades of Neurobiology were unique and were an exciting time for all of us. But the progress being made today in the human genome, in our understanding of how the nervous system works, and in unraveling the mysteries of neurological and neuropsychiatric disorders dwarfs many of the accomplishments of those early years. Society too has made remarkable progress, with women and minorities now making up large portions of our student and post-doctoral populations and increasingly occupying prominent academic positions as well. The grant scene could be better of course, and there are serious challenges to academic excellence being promulgated by grant-dollar counting administrators. Such nuisances can and should be dealt with though, and I plan to continue to do so as long as I maintain my active academic career. I have learned much from my colleagues and mentors. Throughout my career I have tried to emulate Steve and run my laboratory as a "family"; to follow my colleagues Ed Furshpan and Dave Potter and maintain a commitment to excellence in teaching; to give back through service to a field that has given me so much; and to nurture and support our next generations trying to instill in them the same values that I hold to so dearly. Science was fun

in the decades of the 1960s, 1970s, and 1980s, and I suspect we could keep it fun for future decades as well with some serious attention to that aspect of academic life by all of us. Overall though, the good old days are now still seems to ring true to me.

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