

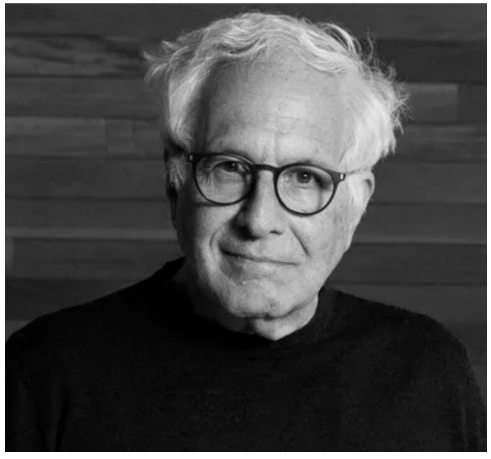
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Jeff W. Lichtman

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Fellow, Harvard Medical School (1980–1982), Washington University School of Medicine (1982–1983)
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Assistant, Associate, Full Professor, Anatomy and Neurobiology, Washington University School Medicine (1985– 2004)
Director, Bakewell Neuroimaging Laboratory, Washington University School of Medicine. (1995–2004)
Director, Neuroscience Graduate Program, Washington University (1992–1997)
Professor of Neurobiology, Harvard University (2004–2010)
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Elected to Phi Beta Kappa, Bowdoin College (1972)
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Nikon Fellow, Marine Biological Laboratory, Woods Hole (1996)
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Dart Scholar, Marine Biological Laboratory, Woods Hole (2003–2005)
Trustee, The Grass Foundation (2012–2016)
Elected Honorary Member of The American Association of Anatomists (2013)
Elected, National Academy of Sciences (2014)
Centenary Medal, University of Concepcion, Chile (2019)
Ariëns Kappers Medal, Royal Netherlands Academy of Arts and Sciences (2026)

Jeff Lichtman's research, beginning with his graduate work, focuses on describing how neuronal circuits are structurally and functionally modified by experience. He is one of the founders of the field now known as connectomics. To visualize neural circuits, his lab developed approaches to render all interacting neuronal elements in brain samples, including generating Brainbow transgenic animals and automated serial-section electron microscopy methods to study human brain tissue. Using these techniques, he and his colleagues have generated synapse-level connectomes of a variety of animals. These include the first full vertebrate connectome, in a fish, and ongoing work to develop the full connectome of a mouse brain. His primary research focus is synapse elimination, the profound alteration in mammalian neuronal connections that occurs during early postnatal life. He and his colleagues have uncovered the way motor neuron activity in early life gives rise to a stable trace (an engram) in the wiring of the neuromuscular system.

Jeff W. Lichtman

Foreword

Neurobiologists know that to learn why an animal's brain works the way it does, it is useful to consider its phylogenetic history. This history is important because an animal's behavioral repertoire is largely shaped by what it has inherited from its ancestors. So, I'm not sure much would be learned by reading the biography of an individual worm from my garden or of a squirrel that lives in the oak tree in my backyard. However, because human brains develop slowly, we are greatly affected by the particulars of our individual lives. These personal histories make us what we are. Of course, these stories are also related to the cultural milieus we find ourselves in and were born into. So, the happenstances that befell our ancestors, the events that continue to affect each of us, and the many people and other living things we interact with all play a role in shaping us. I see the autobiography I've been asked to write in this context—as an exercise in providing the backdrop against which to understand how I became who I am.

Family

My parents were part of the sizable cohort of first- and second-generation Jewish émigrés from Eastern Europe who settled in and around New York City in the late nineteenth and early twentieth centuries. The family name Lichtman sounds German, but family lore passed down to me by my father claimed that my paternal roots were in Spain (Sephardic Jews) rather than in Eastern Europe, where Jewish surnames were more German-sounding (Ashkenazi Jews).

The story was that a nobleman from the Russian Empire and his new bride were on their honeymoon, visiting European cities in search of skilled artisans to furnish the castle where they would live. In a Spanish town, they met a silversmith who designed a beautiful candelabra. They invited him to Russia to illuminate their planned palace. Apparently, the silversmith would only go if additional Jewish men were allowed to accompany him for a 10-man prayer group (a *minyan*). It was said that the prince agreed to transport all these men and their families. All 10 were considered light men (*liktht* is the word for light or candle in Yiddish) and hence the origin of the name. Whether this is a fact or fiction, I can't say. However, given my long-standing professional focus on imaging, much of it with light-based microscopy, I am happy to have that association, fable or not.

What I do know with more certainty is that my paternal great-grandfather, Moses Lichtman, came to the United States in 1890 through the Castle Garden Immigration Center in New York, which was the precursor to Ellis Island. Moses was from the town of Kėdainiai (Yiddish: Keidan), which is in Lithuania, then part of the Russian Empire. Keidan was a well-known Lithuanian Jewish (Litvak) center of learning and commerce. Although I am disconnected from these ancestors, I imagine them as kindred spirits, given the Litvak reputation for rational argument in contrast to the mystical traditions of the other main regional European Jewish Galitzianer tradition. Keidan was overseen by the Radziwiłł noble family, who, unlike some other Lithuanians, welcomed Jewish settlement, establishing a symbiotic relationship that lasted for three centuries. In terms of the story behind "Lichtman," the Radziwiłł family fit the bill. They had multiple castles and were highly cultured and well-traveled, so perhaps the story of a visit to Spain has some truth. Whatever the truth, it now appears unknowable because of what happened in Lithuania 10 years before my birth.

For the last 1,000 years, Lithuania has had a complicated relationship with its borderlands. Before 1795, it was part of a two-century commonwealth with Poland. It then became self-governed but part of the Russian Empire until the tumultuous years of World War II. In 1940, when Nazi Germany began its annexation of Western Europe, a nonaggression pact was drawn up between the Nazis and the Soviet Union, with a secret protocol establishing Soviet eastern and German western spheres of "influence." This pact led the U.S.S.R. to occupy Lithuania and install a communist government, turning it into the Lithuanian Soviet Socialist Republic.

This annexation tested Lithuanian nationalist pride. Rumors spread that the blame for the communist invasion lay with Jews in Lithuania, who were seen as sympathetic to communism. Jewish complicity in the communist takeover was, I think, false, but the lie was in keeping with a more widespread narrative promoted by the Nazis, who claimed that Jews were responsible for Bolshevism and the spread of the communist agenda.

This Big Lie fueled deadly antisemitism in Europe and especially in Lithuania. Things only got worse a year later when Nazi Germany reneged on its nonaggression pact with Russia and occupied Lithuania during World War II. The Lithuanian nationalists viewed the German invasion positively because it liberated them from the short-lived communist rule. These nationalists also found that their antisemitic views of Jews were in line with Nazi ideology.

Once in power, the Nazis encouraged the mass extermination of Lithuanian Jews, mainly at the hands of their fellow countrymen. Most Lithuanian Jews were not deported to concentration or labor camps. Instead, Jewish men, women, and children were rounded up in town after town by non-Jewish Lithuanian citizens, marched to the edge of town,

shot, and buried in mass pits. More than 99 percent of Lithuania's Jews were killed, more than in any other part of the Nazi scourge. In addition, the Lithuanian synagogues were destroyed, and the houses of the slain Jews were appropriated. The Litvak community of scholars, artists, and merchants disappeared from Europe, and so did my family's history.

No doubt my great-grandfather and other Jews from Keidan who emigrated to the United States and Israel 50 years before the Holocaust had reason to leave, but they also appear to have taken pride in their cultural heritage in Keidan. In 1900, 10 years after arriving in the United States, Moses Lichtman proposed founding the Keidaner Association of New York, which operated for about 50 years. It pains me that I have very little else to say about my family's history. My mother's parents came to the United States "to escape the pogroms," presumably in Russia. That my father and mother shared very little about their ancestral homes in Europe was perhaps because their parents and grandparents had little interest in describing the difficult circumstances that led to the move to the United States, leaving so much unsaid. I was imbued with this forward-looking perspective, seeing, as my parents did, each step as a prelude to the next.

I know more about my family members—at least on the paternal side—after they settled in the United States, thanks to an unpublished memoir my father wrote in the last decade of his life. My great-grandfather made a living giving Hebrew lessons to children in Brooklyn. His son (one of nine), my grandfather, was captain of both his high school baseball and football teams and became a dentist. His two children, including my father, were part of the Greatest Generation. They were born before the Great Depression and were upwardly mobile, eventually moving out of New York City and into the suburbs. My father and mother were born, raised, and educated in Brooklyn. In addition to attending elementary and high school in Brooklyn, my father attended Brooklyn College. He earned a medical degree from Long Island College of Medicine, also in Brooklyn. He then did his residency at Kings County Hospital in Brooklyn. My mother was trained as a dietitian and attended Pratt Institute in Clinton Hill, Brooklyn. She helped manage a cafeteria near Kings County Hospital, where she met my father. They eventually married.

Shortly after their marriage, my father was conscripted into the army at the tail end of World War II to serve as a physician. Most of the 100-plus medical school cohort who trained with my father ended up in the South Pacific, but 12 of them, who, like my father, had German-sounding names, were sent to Germany. It was not lost on them that they were mostly Jewish and, by my father's account, had a visceral aversion to going to Germany in 1946. My father was stationed as the medical officer in the small town of Eschwege in northeast Germany. It was the site of a displaced person's camp composed of Holocaust survivors. My father interacted with many of them, and a few became his lifelong friends. His view was that these

survivors were extraordinary and must have had very special traits that allowed them to survive when virtually everyone around them perished.

My parents, as born-and-bred Brooklynites, spent from July 1950 to July 1951 very much out of their element in Salt Lake City, Utah. My father sought mentorship from Maxwell Wintrobe, who was a medical doctor and doctor of philosophy, the eminent hematologist of his generation. Wintrobe came to Salt Lake from Johns Hopkins to establish a new four-year medical school at the University of Utah and to serve as its chair of medicine. In April 1951, Salt Lake City was also my birthplace. According to my father's memoir, he was my last-minute obstetrician. My mother's obstetrician was on a plane to Chicago when she went into labor at the university hospital, and the obstetric resident who was on call that evening was at dinner in a restaurant, but no one knew where. There were no cell phones, of course. My father wrote that my delivery was complicated by a sudden placental abruption, requiring him to cut the umbilical cord before I was delivered. He documented a lot of this by bringing a 35-millimeter camera into the delivery room. My baby photo album begins with several pages of black-and-white shots of me, minutes old, naked on a gurney, and my mother nowhere in sight. This scene fits my sense of my take-charge father. I can only imagine how my mother, a somewhat reserved person, felt about his heroics and his documentation of the events of my birth.

My time in Salt Lake lasted three months. My father's memoir does not explain why the three of us left Utah a year into his fellowship. It does note that Wintrobe was unhappy that he didn't stay the expected two years. I suspect that my mother felt the need to return to New York to aid her recently widowed mother, who was depressed and suicidal, and who took her own life a few years later. Perhaps they also realized they needed some family support in caring for their first infant. And so they, with me in tow, moved back to Brooklyn, where my father took a faculty position at the newly named Downstate Medical Center (also in Brooklyn).

For the next six years, we lived in Brooklyn, adding my younger siblings, Andrew and Susan, whom we called Ange and Sue-Sue. As this is not their biographies, I won't be able to do justice to their very impressive lives. Both became professors: my brother in pathology and immunology at Harvard, and my sister, a painter and chair of the Art Department at Brandeis. As children, we lived in a modest part of the borough of Brooklyn known as the Flatlands, on a street lined with small, narrow one- and two-family homes, separated by double driveways between the neighboring houses. On a trip to New York 50 years later, I revisited the street where I lived. By appearances, I found that almost nothing had changed. Other than new aluminum siding, my house was as I remembered it. Seeing the street, I remembered how much time my brother and I, barely in school, spent playing outside with the 10 or so other kids on the block. We were left to our own devices. We roamed our street unchaperoned, but somehow

knew never to go beyond the corner candy store on Avenue J or the big street on the other side, Avenue K. This style of urban childhood was somewhat like what my father described about his own youth, pointing out how much childhood—and play—changed for the next generation.

If I remember correctly, an Irish-descended barber and his family, and a single, perhaps Italian or Polish, fisherman were our immediate next-door neighbors. Another doctor, a proctologist whose son, Jeffrey, was my friend, lived a few doors down the street. The neighborhood was diverse, with Irish, Polish, and Italian Catholic descendants, as well as Ashkenazi Jews with surnames like Levy, Cohen, and Lichtman. To us as children, these seemed like distinctions without a difference. Similarly, regarding the educational class, we were oblivious. The academic medicine route my father chose was not remunerative. It didn't occur to us that it was surprising that a medical doctor, a barber, and a fisherman were next-door neighbors. Unlike my parents' experiences, my siblings and I don't remember anything akin to antisemitism in our childhoods or even in our adult lives.

Religion in general took a back seat. Although we certainly did not have a Christmas tree, we also did not light candles on Friday to welcome Shabbat. Our nod to Judaism was lighting the menorah candles for the eight days of Chanukah and, of course, getting a present a day for eight days in a row! In retrospect, while I knew well that I was Jewish and was warned by my parents that dangers were lurking for us at every turn, we were a secularized family in a secularized neighborhood. As children, we found this foreboding quite abstract. It is only now that I am beginning to realize that this halcyon era of relative safety, which began in my childhood and extended into most of my adulthood, may have been more the exception than the rule.

Our summers were spent in New Hampshire, in the "country" where my father was camp doctor for two children's camps on beautiful Newfound Lake: Tomahawk on the eastern shore for the boys and, across the lake, Wicosuta, for the girls. I started summering there as an infant. Eventually, my siblings and I became campers. This was a wondrous place for us. By the time I was old enough to be a Cherokee, which was the youngest "bunk" at Tomahawk, I was already familiar with and could name many of the sizable mountains that were in constant view. I loved being outdoors, and unlike my brother, who was freckled and fair-skinned and often had a dollop of Desitin on his nose to keep it from peeling, I tanned to a reasonably dark color each summer.

Tomahawk was an athletically focused camp. Depending on the summer, the head counselor was either the coach of the New York University or Columbia basketball teams (Lou Rossini and Jack Rohan). They brought their varsity squads, who earned their keep as counselors. Most of them were not native New Yorkers, and to my Brooklyn-trained ear, all sounded like they had Southern accents. But that meant they were

from all places south and west of New England, that is, pretty much the rest of the country. They were gifted basketball players and mostly quite tall. A long time later, it occurred to me that there were no black counselors, even though these teams had black players.

Many of the campers came to hone their shooting and dribbling skills, and many seemed to me to be excellent athletes, especially in basketball. I was not one of them. Although I wasn't uncoordinated, I was always at the very short end of the height distribution, making me the least valuable player in things like volleyball and basketball. Also, I was less interested in competitive sports than most kids I knew. I didn't particularly enjoy playing board games either. In retrospect, this may have been because I didn't like losing and realized early on that the only way never to lose is never to play. For whatever reason, I was drawn to less competitive activities, such as collecting stamps and rocks. I started a rock collection at an early age and had befriended a neighbor whose father was a geologist. He supplied his son with uncut semiprecious gems, geodes, crystals, and the like. Some of them were handed down or traded to me, so over time I had a pretty good collection and could name most common rocks and minerals.

New Hampshire was a great place to collect rocks, and my father encouraged me. I remember early mornings when my father would arrange to meet me at sunrise for rock hunting walks. I was already a camper so I would sneak out of my bunk for these little excursions on the camp's grounds. On one of these walks, I looked down and found a brownish hexagonal crystal that, based on its hardness, shape, and other features, I surmised was a crystalline apatite. I was really proud of this find. On another walk with my father, I found a three-inch arrowhead that turned out to be a spearhead because of its larger size. This was quite a find, and the head counselor summoned me to raise the flag at the morning assembly in recognition of my good luck. Unearthing these finds solidified my interest in geology, which continues to this day.

Two decades later, during winter break, when I was visiting home from medical school, I recounted to my father how much these early experiences, and the positive feedback I received for being such a talented observer, had shaped my research interests. He was pleased to hear it and grinned as he told me that he planted both the crystal and the arrowhead for me to find. Although this did not make me happy to hear, I realized I had taken that notion—that I possessed good observational skills—as a lynchpin of my entire professional career. What I don't know is whether this possibly mistaken belief enhanced my observational skills by focusing my attention on observation, or whether, in fact, I was a reasonably good observer to begin with. Was my father trying to show why having that often-neglected skill is valuable? I was too angry to ask him anything when he revealed his trick, and the subject, a sore point for me, was never revisited.

My father was clearly an excellent observer, and particularly of the world of living things. He could recognize many bird species by their songs; he knew the names of all the trees by their leaves and bark; he recognized all the poisonous plants and mushrooms. But he could also name many constellations in the night sky, and he knew many rock names before I did. He gained all this information as a Boy Scout. How he came to be a Boy Scout seems a bit accidental, apparently on a whim when a neighborhood friend in Brooklyn suggested he join. He took to this novel activity and excelled, becoming an Eagle Scout, an Order of the Arrow member, and the summer camp bugler for his division at Ten Mile River Scout Camp on the Delaware River. He was chosen as one of the handful of Brooklyn Boy Scouts who attended the national jamboree in Washington, D.C., where he said he briefly met Franklin D. Roosevelt. By the time he was 16, he was already the nature counselor for the two divisions of the camp with hundreds of scouts learning about nature from him. As the official "naturalist," it was his business to know everything and answer every question every camper had about the natural world. When we, his children, went on walks in the woods with him, this encyclopedic knowledge was nearly always on display. I took for granted that naming things was a way of knowing them, although more recently I have begun to question its validity.

Education

In 1957, when I was in first grade, my brother was in kindergarten, and my sister was about a year old, my parents decided it was time to move out of the city to a suburb with an excellent school system. At least this is what they told us was the reason we were moving to Scarsdale in Westchester County.

Scarsdale was about a 30-mile drive north of my father's job at Downstate Medical Center, and the commute took about an hour. We lived there for 12 years, with one exceptional year, 1962–1963, when we lived in England. My father took a sabbatical there to study genetics, because it was closely related to the blood diseases he studied, particularly sickle cell anemia and thalassemia. In England, he worked at the Galton Lab at University College London (UCL) with Sir Lionel Penrose, an expert in human genetics and the application of statistical methods, to better understand heredity. In 1963, when my father was there, the lab was renamed from "The Francis Galton Laboratory of National Eugenics" to the "Department of Human Genetics and Biometry." Penrose was responsible for this move away from the use of "eugenics," which was intertwined with now-debunked racial theories promoted by Nazis and others. He was the father of several other well-known, exceptional British Penroses, including Sir Roger Penrose (Nobel Prize in Physics 2020), Jonathan

Penrose (British chess grandmaster), Oliver Penrose (another physicist), and Shirley Hodgson (cancer geneticist). Ironically, genes may have played a role in that family's intellectual gifts.

My year during that sabbatical was spent in Purley, a commuter town 13 miles south of central London. My brother and I were enrolled in Saint Anne's Preparatory, a now-defunct boys' school, requiring us to wear gray blazers, short pants, high socks with a green garter, and a red cap with the school insignia. I mainly remember the "Scriptures" class and what seemed to me the inedible lunches of rice pudding that we had to eat or get caned. Corporal punishment, meted out with a bamboo stick on the palm or, if you were very naughty, on the backside, kept me on my toes. I was happy to be back in the states after a year.

Looking back at my elementary, junior, and senior high school years in Scarsdale, this period seems like a rather short time. Still, in the context of my childhood, it was 75 percent of my life. So it had outsize importance in all that followed. Inadvertently, perhaps, a small gesture my father made during my early years in the suburbs was responsible for the direction of my future profession.

My father, like all medical students at that time, was required to purchase a microscope to do urinalyses, blood smears, Gram stains for bacteria, and the like. When his research interests in hematology allowed him to purchase a research-grade scope, he put his med school Leica monocular compound microscope on a desk in the bedroom that my brother and I shared. I do not remember him giving us any instructions on how to use the thing. But at some point, I began putting samples on slides and examining them using several objectives. The illumination was provided either by a desk lamp (at night) or the sun through the tiltable mirror under the microscope's stage.

I believe I spent a lot of time looking at things. I was surprised by the woven fibers in paper, and by how much less precise the ink in printed letters was than the naked eye could appreciate. My attention eventually drifted to living things like bread mold, small insects, skin, hair, and various bodily secretions. At some point, I set up a rectangular rubber storage container on the windowsill with some soil, leaves, and water. It began to smell, and my mother made me cover it, which I discovered made the smell far worse when I opened it, because of the growth of anaerobic organisms. But inside, I discovered an amazing new world. I found a book that showed what paramecia, amoebae, rotifers, and various algae looked like. Without much supervision, I began to be able to recognize these creatures by their scientific names, although I wasn't really interested in the names as such, because there was no one to impress with the names of invisible things. More important for me, however, were the many hours I spent following them as they darted forward, backed up, paused, and seemed to be perpetually exploring. I began to think of these protists as living, behaving creatures

with motives and agency. This idea stuck with me ever since. My view of neurons in the brain is as a society of single-celled creatures living in a weird, dark pond, spending their time doing what they must to survive in a competitive ecosystem.

When I moved into my own room in the former attic of our house in the seventh grade, I took the scope with me. I began using the oil-immersion lens, which provided higher numerical aperture, brighter images, and finer resolution. At the time, I could clearly see that the oil between the objective and the coverslip made the microscope images pop, but I didn't know why. Much later, when I began teaching microscopy and building microscopes, I did a deep dive and fully understood the subtleties of how optical resolution and light-gathering were related to oil.

I don't remember anyone having much interest in my interests when I was a child and adolescent, including in my use of the microscope. I am not sure what I thought I was doing, spending time looking at things. Probably, I thought this was a good way to waste time and avoid doing homework. I didn't think it was a hobby like rock collecting (even though it was). I didn't think it was a useful way to learn about biology (even though it was). I never thought having a skill with a microscope was something to aspire to. It seemed totally unrelated to the skills kids honed by shooting hoops, juggling, riding a unicycle, and speaking a foreign language. It never occurred to me to take advantage of this interest, for example, by writing about microscopy in my college applications. At the time, I didn't think it was any more than a pastime or perhaps, as noted, a waste of time. Growing up, I often felt sidetracked by curiosity, and this tool slaked that urge to avoid schoolwork in favor of something more interesting.

In school, I was a mediocre student. I struggled with some things other kids found easy, but at the same time, some things that other kids found challenging seemed easy to me. For example, in elementary school, the SRA (Science Research Associates) Reading Labs were used to improve our reading skills, with each student progressing at their own pace. After initial screening, each of us was instructed to read pieces at a certain color level. As we mastered that material, we then progressed to higher colors. I think purple was the absolute top, and red, the bottom. I knew where I stood by looking at the colors of my classmates' book covers. The smart kids were reading in the greens and blues while I was mired in the orange and browns near the bottom of the color scale, and I don't remember ever moving up. But at the same time, my second- and third-grade teacher, Miss Morris, kept giving me math workbooks used by third-, fourth- and, finally, fifth-graders. I remember some of the big fifth-graders pointing and smirking when I, as a second-grader, walked to school with the same workbook they were using.

Things were much the same in junior high. My English teacher told me she would not recommend me for the honors English track in high school,

where most of my friends were headed, because I was a terrible speller. I hoped she thought this was a shame (I remember her sad face) because I seemed to have good thinking ability, but how could one write if one couldn't spell?

And in high school, it was the same: In French, we began the year just speaking French, and my teacher, Mrs. Mantz, loved my accent and fluency; I think I was her favorite student. About a month in, we had our first *dictée*, where we had to write in French what she said. I got a C double minus (C−−) with "???" affixed. She was incredulous and asked me what happened. I remember saying I didn't know, which was the truth. As the year rolled on, it became clear that whatever happened in that first dictation was no fluke; I couldn't spell French any better than I could spell English words, and her view of my talent for foreign languages dropped precipitously. I ended the year with a C+ in French, the only C grade I got in high school.

I was convinced that I was stupid. In the standard nonhonors English classes, my teachers thought I was cheating or copying when I spoke up, putting on airs with fancy words and ideas, even though I was clearly illiterate. At the time, there was no such thing as dyslexia, just smart and dumb kids. It certainly didn't occur to me that there was something other than lack of smarts and perhaps profound laziness that was holding me back. I ended high school as a barely B+ student (87 percent). Many of my friends were much better students and headed to Ivy League schools.

My college counselor, Mr. Reed, if I recall correctly, was a sympathetic guy who had gone to Bates College in Maine. He suggested I consider Bowdoin, a good school in Maine. He thought I might have a chance because, at the time, it was strictly for men, which lowered demand a bit. I applied early decision and was deferred. My college entrance test scores (SATs) were okay but not great, so I retook them, this time with some studying. On the second try, my scores, surprisingly, were excellent, which I guess was enough to convince Bowdoin to take a chance on me. I am glad they did.

The only other noteworthy thing in high school (besides being president of the Stamp Club) was an extracurricular interest in writing and producing stop-motion animated films. Together with two friends—Neil, who went on to Harvard and became a pediatrician, and Michael, who went to Brandeis and became an architect—we wrote and produced films. Ironically, given my eventual career, our largest production was an animated film of a professor giving a lecture. The professor was made from painted cardboard with jointed elbows and knees. In the film, he was giving an absurd lecture while birds, planes, and other airborne objects flew by in the classroom. I wrote the script and helped with the arts and crafts. Neil was the voice of the professor, and Mike was the film's director and cameraman using Super 8 film. This all seemed so hilarious and a

joyful waste of time. I don't think we had any interest in showing this film; we just wanted to make it. But if memory serves, it was pretty good. This production was in the same spirit as *Monty Python's Flying Circus*, but when I recently checked the dates, I discovered our efforts preceded theirs by at least a year or two, because the Monty Python troupe didn't release any of their fantastical productions until we were in college. Eventually, filming time-lapse fluorescence and three-dimensional (3D) computer-assisted movies became a mainstay of my professional life.

The four years of college seemed to go by much faster than the four years of high school. I took to heart the liberal arts dictum to go broad, not deep. Now, I am less inclined to this approach, but then I was completely unsure what I was destined to become. I felt there was some parental pressure to follow in my father's footsteps and become a physician, but I was less inclined toward service and more toward creation. I imagined being a writer (despite my spelling) or a musician and composer. I had played piano since grade school, and as I was leaving high school, my interest in "writing" music surged. Actually, I was only able to compose by improvising and refining it until it became a set piece I could play fluently. I never wrote my compositions down; I just memorized them, perhaps exposing another weakness in my ability to use the medium of writing. I took music theory classes intended for students planning to major in music. I also took the introductory philosophy course for prospective majors, along with additional English and many science courses.

There was little doubt that science material was far easier for me than music, English, or philosophy, all of which demanded a good deal of writing and reading. I saw this as a challenge: doing something hard rather than something that seemed easy. My mind began to change on this point, however, as I absorbed what my education thus far had taught me: I am, perhaps like most people, intrinsically better at some things than others. If one wants to make progress, maybe the best thing to do is lean into what one finds easy and keep pushing until one reaches a point at which one is challenged, and that is where real progress takes place. Leaning in on something you find challenging from the outset will put you at a permanent disadvantage compared with others pursuing the same area who find it easygoing.

I saw this firsthand at Bowdoin. I was composing a lot of piano music and played these pieces for my friends. One of these friends, Hilliard, who became an art historian and museum curator, was a skilled classical pianist and had perfect pitch. After I performed one of my longer pieces, he immediately sat down and played the whole thing again, effortlessly, it seemed. He told me he wasn't very good at improvising because as soon as he started improvising, something he knew from his classical training would pop into his head and basically take over the improvisation. I realized that my creativity combined with his innate skill would make for a far

better composer than I could ever be alone, and that combination was why great composers are great and why I was not.

In the literary arts, I fared no better. I talked my way onto the board of *The Quill*, Bowdoin's literary magazine, and by the time I was a senior, I was editor in chief. Truth be told, my literary aspirations far exceeded my skill. Not being a good writer went hand in hand with not being a good reader. It took me longer to read than it did most people I knew. Reading a novel in her spare time takes my wife, Linda, about a week; for me, it takes months. I read every word, but it is painstaking work for me. For Linda, the language enters her mind much more quickly; she has even told me she doesn't need to hear the words in her head to understand what she is reading. That approach to reading is profoundly alien to me.

Speaking of my spouse, I recall interviewing classmates who wanted to be on the *Quill*'s editorial board and found that many of them were far more qualified than I was. One female student interviewed for a board position. At that time, women had just begun to be admitted to Bowdoin, 177 years after its founding in 1794. I asked all the typical questions, like, "What do you find interesting in literature?" To that question, she replied that she was studying Milton, and I said, astutely, "Milton Berle?" That gives a sense of my illiteracy, and she did not spare me, telling me that I was an idiot. I was charmed, and nine years later, Linda Tucci agreed to marry me. She became an accomplished journalist and is now executive editor at a business-to-business technology media company.

As arts and humanities professors began forcefully steering me away from areas like music, English, and philosophy, I was encouraged by the science faculty. Science was the only thing that I seemed to have some aptitude for, and my view, as mentioned above, was to lean into the easy. I gravitated toward labs with microscopes and courses that included microscopy. I took a botany course and learned a great deal about algae. I took a two-semester independent study in biophysics and learned about fluorescence and the algal protist Chlamydomonas.

In that project, I was trying to see whether I could distinguish *in vivo* RNA and DNA staining using the metachromatic dye acridine orange. When the acridine molecule intercalates into the double-stranded DNA polymer, it fluoresces green, with an emission peak around 530 nm and excitation peak at 502 nm. However, acridine orange binds to single-stranded RNA via electrostatic attraction, and many acridine molecules stack to form an aggregate that fluoresces red, with an emission peak around 650 nm (excitation often shifts to ~460 nm). This shift to a larger excitation-emission gap is explained by the collective behavior of acridine dye aggregates, which act as excimers. I had a steep climb to learn all this brand-new (to me) photo-physics. Additionally, it was my first experience observing multiple fluorescent colors in the same biological sample using specialized dyes, a subject that kept me busy for the next 50 years.

Many years after this first foray into fluorescence, my dear colleague José Conchello and I wrote a primer on "Fluorescence Microscopy" for *Nature Methods* (Lichtman and Conchello 2005). That is one of my most cited papers.

It was in college classes and labs that I began to realize how clumsy most microscope users were. My classmates seemed uncomfortable with the location of the knobs, adjusting the eye pieces, finding the relevant part of a slide, and so on. Of course, for many of them, this was their first experience with microscopy. My early childhood experience with microscopes, thanks to my father, gave me a leg up and set me on a path on which things were easier.

I was in college at a tumultuous time. The United States was deeply engaged in the Vietnam (undeclared) War. Students like me, who had matriculated in 1969, were deferred from the draft (2S) as long as we remained in good standing. This meant that our studies took on increased importance. For the other potential premed students and me, our Waterloo was the first-year organic chemistry course. This was a hard course, and many of us saw it as the establishment's way to weed out weaker students from the medical profession. As this class took place in my first year at a new school, I had no idea whether I might be weeded out of medicine or, worse, out of college. Failing might mean obligatory military service. I could not avoid this yearlong ordeal because it was an absolute requirement for admission to medical school. My parents were paying for my education, and they expected me to consider a career in medicine. Suddenly, I felt that my education was far more consequential than it had seemed in high school. I was anxious. I have since wondered about the wisdom of having young people face so much stress during their education, as it tamps down their ability to think creatively. I am especially doubtful about the need for course requirements that are not essential to a particular career path. Courses that cull future physicians based on innate skill in organic chemistry or calculus seem misguided to me. I see education as quite distinct from evaluation. When schools see their role as culling the wishful students in favor of the worthy ones based on "hard" classes, they are not educating.

This issue became critical for me in the second semester of organic chemistry, which seemed much harder than the first, and my midterm grades were poor. I found it difficult to read the chemical formulations and to comprehend this written logic. As it turned out, my fate was determined by tragic events taking place hundreds of miles away when four undergrads at Kent State University, including a freshman like me, were shot to death on May 4, 1970, by the Ohio National Guard while protesting the expansion of the Vietnam War into Cambodia. The war's expansion and the killings in Ohio led to strikes and shutdowns of many of the nation's colleges and universities. Bowdoin officially closed its doors a few days

after the killings. Final exams were not given. Students who were passing their classes were given credit for that semester's work. I squeaked by and dodged a bullet while fully aware that Kent State freshman Allison Krause was not so lucky.

As I took more advanced classes, my grades improved, and I became an excellent student at Bowdoin thereafter. I did my biology major research project with Professor John Howland, a biochemist. As I remember it, my role was to homogenize rodent livers and assay the effects of various uncouplers of oxidative phosphorylation. It was not my cup of tea, but I was proficient enough in lab technique that my results were incorporated into a not especially impactful publication (Howland, Lichtman, and Settlemire 1973).

One highlight of my time at Bowdoin was an amazing summer spent with a bunch of Bowdoin students, including my brother, working on the Androscoggin River. We analyzed water quality along the river from its entrance into the state in Gilead, Maine, to its mouth at Merrymeeting Bay, covering about 120 miles. We were interested in seeing how the paper mills that processed Maine timber and discharged waste into the river affected water quality.

We conducted many tests, including dropping fluorescent chemicals into the river upstream and then assaying them downstream to measure flow. We also used agar plates to observe bacterial growth and measured the water's turbidity, temperature, and pH. We traveled mostly in a Volkswagen van. One night, it collided with a moose; neither the moose nor the humans were injured, though the van sustained a sizable dent.

This was my first foray into collaborative science, in which team members were assigned different tasks, and the results were compiled into a report we submitted to the state of Maine, which I believe funded the project. Richard Cohen, who was a year ahead of me after graduating, went to Johns Hopkins for medical school and became the head of vascular biology at Boston University, was our leader. The entire experience was fun. The water quality of the Androscoggin has improved remarkably now, but I am not sure our efforts had anything to do with it.

At the end of my junior year, I began to think about next steps. Research in biology was clearly where I wanted to head. However, I still felt some pressure from my parents to consider medical school. As I was looking at potential programs, I saw that Washington University Medical School had recently instituted a schoolwide combined medical doctor–doctorate in philosophy program. I think it was one of two in the country at the time. I knew nothing about Washington University nor its Saint Louis home. But I was intrigued that the entire education was funded, including a stipend for living expenses during medical school. My father told me that it was a great medical school and that he read the *American Journal of Medicine* ("the green journal") because it printed Clinicopathologic Conference Cases

from the Medicine and Pathology Departments there, which he found quite instructive, given his interests.

His seal of approval, the fact that I would be far from home, and, if accepted, that I would no longer be financially dependent on my parents, motivated me. I applied to their Medical Scientist Training Program (MSTP), which used an interview process that differed from those at graduate or medical schools. In October, I was flown in for a day of one-on-one meetings with faculty, an interview with the medical school dean of admissions, and, notably, the "group" interview, in which research faculty members made up the group and I was the interviewee. In that interview, I was ushered into a reasonably large room and seated at the head of a long table with maybe 18 faculty staring at me. They began asking questions clockwise, starting from my left.

The questions were, as best I can recall, mainly about science, which was good, I thought. I noticed that as soon as I showed that I could answer a question, I was interrupted by the next faculty member's question, which was frustrating because I wanted to launch into some long disquisitions showing my "depth" of knowledge.

Now, I only remember a few of these questions, particularly one from Stephen Kinsky, a professor of pharmacology, who sat at the back end of the table and faced me directly. He said simply: "Who is Vane?" Because I had done a summer project on prostaglandins and had written about it in my application, I vaguely recalled that Vane had shown that aspirin blocked prostaglandin synthesis and said so. At that time, the full consequences of this discovery were not known, as it was less than two years old. I could see people were surprised by the question's bluntness and perhaps by my ability to answer it. Eleven years later, Vane won a Nobel Prize, but back then, he was not too well known.

So, I felt good, but that feeling didn't last long. The next interviewer asked something I could not answer (and no longer remember). When I stumbled, the interview host said, "Thank you very much; you are dismissed." I stood up to leave a bit dazed and rattled, realizing that the final third of the people at the table never got to ask a question. I was demoralized, but a few weeks later, in November, I received an acceptance letter.

Although I had applied to other regular medical doctorate programs at other medical schools, I sent my regrets before hearing from most of them because I knew the MSTP program was the right fit for me. That said, I had interviewed at Yale and Harvard, and my mother wanted me to see if I got in, perhaps because of the proximity or because these name brands were more familiar than Washington University in Saint Louis. I got into Yale's medical school, and she said, offhandedly, that I would obviously go there instead of a school in Missouri. At that point, I sent my regrets to Yale and Harvard (which hadn't yet made its decision)

to end this line of thought. I was more interested in financial independence and the opportunity to do graduate-level research than the school's name. It turned out that in neuroscience, I could not have been in a better place than Washington University, and I have never regretted for a moment my time there, which ultimately spanned 30 years.

The first summer, before first-year classes began, I rotated with Steve Kinsky, the person who asked the last question I answered at my group interview. He worked on liposomes, which are human-made, spherical, membrane-enclosed vesicles. As a pharmacologist, he understood that they might be therapeutically beneficial for drug delivery. His concern was the immunogenic reactions that might limit their efficacy. I worked on some aspects of the liposome trafficking during their complement-mediated destruction by the immune system. After the rotation, he offered me a position, but it didn't feel like the right lab, as it was more biochemical than cellular-focused. I found molecules a bit abstract; they all had names, but their physical properties and how these related to their functions were obscure to me. In contrast, I was comfortable with cells, having grown up watching them from a young age. Also, microscopes were not a key part of his armamentarium. However, imaging the trafficking of natural liposomes (i.e., synaptic vesicles) turned out to be the way I began my independent career 12 years after this rotation (Lichtman, Wilkinson, and Rich 1985). My next rotation would have to wait until the end of the first year of medical school.

Medical school, at least at the outset, was a grind. It felt like a return to the worst aspects of college premed science courses: large classes, lots of memorization, and less-than-gifted teachers, with the added challenge that now it was all day, every weekday, rather than the more leisurely pace of undergraduate classes.

To make matters worse, I was operating under several false assumptions. For example, I assumed that being from the Northeast, I would somehow have an intellectual advantage over the mostly Midwestern students in my medical school class. Perhaps because of their slower speaking style, I assumed they would be slower in other ways. Reality set in after the first exam, which was in immunology. The professor was pretty good, and I learned a lot from listening to his lectures. In fact, I got 96 percent on the first exam. When the grades were posted, I found that I was in the bottom 10th percentile because most of the class got 100 percent.

In many of my other classes, I was also quite undistinguished. Whether this was because I was undisciplined and lazy, which is what I had always thought, or, in retrospect, whether this was a consequence of my poor reading skills, I don't know. But my subpar performance was noted by the head of the Division of Biology and Biomedical Sciences (DBBS), in which the new MSTP program was situated. Professor Luis Glaser, a biochemist trained by the Coris, was a kindly man but told me that much was expected

from the small cohort of MSTP students that had the good fortune to be matriculated (five in total my year, I think) at Washington University. He implied somewhat gently, but unambiguously, that I needed to improve.

I realized that my performance was directly related to the professor's teaching ability, suggesting I was getting more from the lecture than from the textbooks. So, not surprisingly, the one bright spot in my first year was neurobiology, taught by a superb teacher, Dale Purves, a medical doctor (see volume 11). He had just joined the faculty as an assistant professor in the Department of Physiology and Biophysics. A summa cum laude graduate of Yale, he went on to Harvard Medical School, where he began training in surgery, switched to neurosurgery, but quickly settled on basic neurobiology research as his calling (Purves 2020). He was trained by John Nicholls and Stephen Kuffler in the neurobiology department at Harvard Medical School. Then he did additional work with Burt Sackmann in Sir Bernard Katz's lab at UCL. His new job required him to teach the fundamentals of cellular neuroscience, particularly the three Ps: the resting potential, the action potential, and synaptic potentials.

Electrophysiology was new ground for me. My grounding in physics, especially outside optics, was very weak, so electrical concepts such as resistance, current, voltage, and capacitance were nearly unknown to me. In college, instead of taking the standard introductory physics course, I chose a yearlong biophysics research program, as discussed earlier. In addition, quantitative concepts related to electrochemical gradients, as embodied in the Nernst and the Goldman Hodgkin Katz equations, were alien territory. But the underlying physical principles were so rigorous and, with some work, accessible enough that I found in this area something that eluded me in all my studies thus far: a physical conceptual framework that explained biological phenomena all the way down to the deepest level of atoms or, more accurately, the level of individual ions.

Understanding the movements of Na^+, K^+, Cl^-, and Ca^{++} across membranes seemed sufficient to explain everything the brain did! I fell in love with this area. Dale presented this material so well that a textbook was unnecessary, and it all made sense! That spring, based entirely on Dale's lecture ability, I asked him if I could rotate into his lab. It is hard to believe now, but I really knew next to nothing about his research, and my ignorance was obvious during his interview with me. The only question I remember him asking me was, "How are your hands?" I remember looking down at my hands, not understanding what he was driving at, and when he explained that he was asking about surgical dexterity, I said, "I play piano."

At that time, I had never done anything like dissections, especially nothing like those performed under a stereo microscope. Not surprisingly, he was quite leery to take me on, having been advised that postdocs would be more reliable, but according to Dale's memoir (Purves 2020), the chair of

the Physiology and Biophysics Department Cuy Hunt (see volume 5) told him that my acceptance to the MSTP program was a reliable indicator that I couldn't be too bad and therefore that I probably would be okay. And so began a long and wonderful relationship with Dale Purves. When I began working in his lab, I told my father about Dale, and it was his turn to be a bit leery: He said a junior faculty member is beginning a steep climb in academia, and they may need to put their careers ahead of their students'. In my father's view, it would be safer for me to choose the lab of someone who was already much further along. I ignored this advice, just as Dale ignored the advice that an unseasoned grad student is inappropriate for a starting assistant professor's lab.

In my first summer in Dale Purves's lab, I found it nearly empty: He had a technician and me. He had truly just started over the past academic year and was busy conducting experiments. He had an electrophysiology rig on a thick marble table, elevated by small tires mounted horizontally for vibration isolation. I put together a second table in the same room that, pretty much, was an exact match of his. These electrophysiology rigs were designed for intracellular recording using what is now called "sharp" electrodes (at the time, patch clamping was not yet in use, so impaling neurons with very fine-tipped glass microelectrodes was the best way to assay their membrane potential). The rigs also included means to stimulate nerves through suction electrodes and to record compound action potentials from nerve bundles with a second suction electrode.

Dale had been working on his first project during the academic year on the superior cervical ganglion (SCG) of the guinea pig. He was analyzing what happened to the innervation from the spinal cord to ganglion neurons after the drive to the ganglion cell's peripheral smooth muscle targets was interrupted by cutting the ganglion cell's outgoing axon. By the time I came to the lab, he had made a pretty strong case, although not yet published, that about three days after axotomizing the output of these cells, their synaptic input began to weaken, meaning that neurons disconnected from their downstream targets were losing their upstream input. This suggested some linkage between multiple levels in a synaptic relay. I could already see the trajectory of this interesting perspective. For my rotation project, he asked me to develop an approach to visualize these axotomized cells by intracellular injection of a visible dye.

Dale had previously worked with a great cellular anatomist, Uel "Jack" McMahan, who had performed fluorescent dye injections using Procion yellow and was beginning to work with horseradish peroxidase (HRP). This latter new approach was to inject HRP, which, by virtue of its enzymatic reaction with a benzidine substrate that could pass indiscriminately across cell membranes, could generate far more colored product molecules than the number of molecules of an intracellularly injected dye. Seeing individual neurons in a microscope sounded great to me, so I set to work on getting

HRP injection working. This approach worked well, and I began to analyze the appearance of axotomized versus control SCG cells. I quickly discovered that axotomized neurons had stunted dendrites that ended in bulbs. I used a camera lucida on the microscope to trace these odd-looking neurons with dendritic "retraction bulbs." My summer project was complete, and Dale was pleased with this useful additional data for the paper.

I returned to med school for my second year, happy with the rotation and committed to pursuing my doctorate with him. During winter break, he told me that the paper had been accepted by the *Journal of Physiology* and that several of my tracings were included as figures. I was excited about this because the intracellular HRP injection was the first use of this new technique in the mammalian nervous system, and I knew that I was responsible. When the paper came out, I saw that I was acknowledged for doing some of the injections, but he was the sole author. I told my father about this, and he reminded me of the warning he gave about the challenges of working for a young investigator. One of the challenges, however, was with a peculiarity of the *Journal of Physiology*, which, back then, required that the authors be listed alphabetically. It is why we think of famous publishing duos like Hubel and Wiesel, del Castillo and Katz, Katz and Miledi, and my favorite quad team: Aguilar, Bisby, Cooper, and Diamond, published in those orders. I knew that "Lichtman and Purves" would not accurately represent his enormous yearlong versus my very brief summer effort. So, again, I did not heed my father's warning and decided not to make an issue out of my nonauthorship.

This research on axotomy taught me a big lesson: Synapses can be lost through changes in the postsynaptic cell's state. This idea permeated much of my subsequent work on synapse elimination that continues to this day.

The rest of that second year of medical was uneventful except that I failed obstetrics and gynecology. This was a classroom course with poor teachers and an impenetrable textbook—a perfect storm for me. I had to repeat the class during my hiatus the next year when my doctoral work began. Very embarrassing, but I passed the second time around.

When I rejoined the lab after year two of medical school in the summer of 1975, I had to decide on a thesis project. I would stay in the lab until the thesis was complete and then, years later, rejoin medical school and a class of students whom I had not met previously. To help me start a project, Dale showed me a letter he had received from János Szentágothai (1912–1994), the great Hungarian neuroanatomist, introducing Dale to a novel preparation. In this brief letter was a sketch of the neck of a rat exposing a triangular region of connective tissue bordered laterally by the lingual nerve and medially by the pair of salivary ducts for the submandibular and sublingual salivary glands. The ducts crossed the lingual nerve at the apex of the triangle. As I remember it, Szentágothai drew small clusters of neurons in the connective tissue and explained that these were the parasympathetic

ganglion cells that controlled salivation through the two nearby salivary glands. Apparently, no one had studied these cells previously, and Dale thought they would make a good place for a thesis project.

I am grateful that he gave me first dibs on what turned out to be the thing that set me on my career path. I practiced a bit and was able to do the dissection easily. I also found that I could record from these nerve cells with little trouble and activate them by stimulating the lingual nerve, which was the path the preganglionic axons took to the scattered ganglion cells through many small nerve branches that exited the large, mostly sensory lingual nerve. Given their sparse distribution within this triangular connective tissue sheath, the cells were difficult to find. To make intracellular recording easier, I procured an inverted microscope from Cuy Hunt and built a small stage with a coverslip bottom and a magnetic rim to pin the triangle in place using insect pins attached to tiny magnets. Now I could see clusters of cells clearly, and with the use of differential interference contrast (DIC) optics, I had an easy time picking out cells to study.

Many of the ganglion cell clusters were only one cell thick, providing me with remarkable optical access to mammalian neurons. Dale suggested that I see whether the synaptic weakening he had described in axotomized sympathetic neurons also occurred in parasympathetic neurons. This seemed like a good plan, and in reasonably short order, I could see that the same phenomenon was occurring in a parasympathetic ganglion. I felt this was a bit derivative and told him so. To me, it didn't seem like much of an insight. He agreed and next suggested that I see whether the phenomenon of "denervation supersensitivity," in which postsynaptic neurotransmitter receptors distribute themselves all across the membrane of a muscle cell after it loses its excitatory synaptic innervation. This phenomenon had been demonstrated in a year earlier in denervated autonomic neurons in frogs. The question now was whether this was also true for mammalian neurons.

This was a more challenging project because it required iontophoresis of acetylcholine onto the surfaces of denervated and normal ganglion cells while doing intracellular recording from the same cells. With some effort, I got this up and running and obtained results suggesting that the same phenomenon was occurring in mammalian neurons. But again, I was unsatisfied as it seemed too incremental for a thesis project. I again told Dale that this was not my cup of tea. I had already spent six months trying these things out. He was exasperated and asked me if I had any ideas. I did have one. When recording from these ganglion cells, I noticed that many were singly innervated, meaning a single axonal input strongly drove them. I knew this because, when recording from the cells for the first two projects, the response to nerve stimulation was all-or-none: At one stimulation voltage, the cell showed no depolarization, and a slight increase in lingual nerve voltage elicited a suprathreshold response with no intermediate synaptic response.

I found this interesting because I knew from Dale's work on the SCG, whose cells were multiply innervated by around 12 axons, that increasing the strength of stimulus to the nerve gave rise to progressively larger postsynaptic responses. I also remembered a drawing in a paper by McMahan and Kuffler (1971) showing a single axon making multiple synapses on an amphibian autonomic neuron, and the paper making the point that this arrangement was akin to a neuromuscular junction, which was also singly innervated by one axon that established multiple nearby synapses at an endplate. I also knew of a recent paper by Paul Redfern (1970) showing that in distinction to singly innervated adult neuromuscular junctions, newborn neuromuscular junctions were multiply innervated, and in rats, this transition occurred in the first two postnatal weeks.

Putting all this together, I suggested that it would be interesting to check if neurons in the submandibular ganglion also transitioned from multiple to single innervation during development. Dale seemed okay with the idea, and I think it was the next day that I got a young rat pup to record the synaptic potentials from the immature submandibular neurons. It only took a few minutes to realize that neonatal submandibular ganglion cells were innervated very differently from adult cells. They were innervated by many axons, and many of these inputs were much weaker than the suprathreshold input in adults.

As I was recording, Dale walked by, and I was excited to show him this result. His reaction surprised me because he seemed less than impressed and said something to the effect of "What would this show?" I was sure this was an interesting result, but I wasn't yet sure why. I went home that day thinking about his important question: What would this show? I have spent more than 50 years grappling with that question. The next morning, Dale approached me, and apparently, he had also spent some of the previous evening thinking about this. He said that on reflection, he thought this was an interesting project, and I should continue with it. So, we were on the same page again.

In retrospect, I have considered why I thought this was a more interesting project than the ones he had suggested. After all, this project was an extension of the work of Redfern to a different part of the nervous system and was analogous to Dale's suggestions, so was this actually a better project? One possibility is that it wasn't. Rather, it was just more interesting to me because I thought of it. I am sure my ego played a part. But I had no trouble expounding on why this find was potentially very interesting. For me, the critical matter was that humans are, neurologically speaking, profoundly immature at birth and depend on experience to develop language and motor skills and to craft a functional nervous system. I already knew that Homo sapiens is an outlier species, that most animals innately know much of the behavioral repertoire they will need. Humans, in contrast, are obligatory learners and require experience to

generate a nervous system that is fit for the environment they are born into. The neurobiological underpinnings of that difference were poorly understood, and frankly, still are. The fact that, in a mammal, the wiring diagram might change in early postnatal life seemed like an intriguing avenue to pursue, and I have stuck to my guns and continued this line of inquiry ever since.

Research

In Dale's lab, I flourished. He had a highly efficient work style that I tried to emulate, not realizing how unusual it was. He kept strict hours. He came to the lab at a quarter to nine and left at a quarter to six every weekday, and also worked a half day each Saturday. During the workday, we took few, if any, breaks and at the outset, at least, talked rarely. We came to work, and work we did! The day began with each of us preparing our dissections, making Ringer solution, and pulling pipettes for recording. Once everything was ready, we went to our rigs to collect data. In addition to my thesis work, I participated in several collaborative projects with Dale. In many cases, Dale wanted recordings from 200 neurons for each analysis to be included in the eventual publication. To accomplish this, we split the work between his rig and mine. We used xeroxed worksheets that allowed each neuron to be marked with boxes to fill in all relevant data. The pre-computer age meant that all of this was handwritten. In a typical day, we would each collect data on 20 neurons, so that in one week, we could be done with 200 cells and move on to the next experiment the following week.

As I remember it, more specialized data collection, such as electron microscopy, was worked on at the end of the day or on weekends. I quickly realized that I was not nearly as good as Dale at preparing my sample by dissection, nor at getting good "sticks" of neurons. We had a strict criterion for what constituted a suitable resting potential for a cell to be counted. So, I began coming into lab two hours earlier than he. I was always a "morning person," so coming in a little before 7 a.m. suited me fine. This efficient pipeline meant that we were churning out papers at a good clip. Indeed, what often takes the longest for me now is paper writing, but this was done in the evening by Dale. I wondered if he ever slept. For this reason, paper submission also came shortly after the final data acquisition was complete, because he was writing as we were collecting data. The only time to really relax was the lunch break, which was at the same time each day in a lunchroom where many of the faculty gathered, and I usually joined him. This was a treat for me, as it gave me an opportunity to listen in on how faculty thought and talked about things, from gossip to politics to science. Consistent with the orderly way we collected data, Dale's lunch was a white bread sandwich, a 7UP, and a Reese's Peanut Butter Cup two-pack, the same thing, every day.

My work on the submandibular ganglion cells was taking shape. I showed that over the first postnatal month, the number of inputs per ganglion cell was dropping, and by a month, almost all the cells had one suprathreshold input, and about 20 percent had two or more, rarely three or four inputs, but these were subthreshold for the vast majority of cells. Although I was taught about temporal and spatial summation of weak synapses, in this ganglion, the weak synapses played no obvious role because I couldn't stimulate them at high enough frequency to summate above threshold; they would just fatigue. I took this to mean that nature needs to be good enough to achieve the desired outcome, not perfect, to remove small amounts of synaptic "noise." This idea has stuck with me in all my more recent work.

I also showed that these cells rarely had dendrites but did have small, spine-like protrusions from the cell soma where synapses were located. I used a dye that I read about to stain the axonal terminals. Zinc-Iodide Osmium had some specific affinity for cholinergic axons and labeled these axons black. Staining the adult ganglion showed a number of black-stained axons and ganglion cells that were encased in 30–50 black varicosities (boutons). I took these swellings to be the release sites of a preganglionic axon originating in a brainstem nucleus and terminating on the cell body of a ganglion cell.

The same staining approach, however, gave a different result in newborn rat pups. In the neonate, I saw many more stained axons, but the number of boutons was much reduced. I confirmed this with electron microscopy. The conclusion I reached was that as axonal inputs were eliminated, the remaining axon added more synapses, more than compensating for the lost connections. I told Dale I thought this was ready to be published, and he agreed. He suggested that this be published as a "note" to *Nature*, which was the short-form publication in this widely read journal. He also said that this was a solo effort, and I could publish this without him as – coauthor. Even as green as I was, I knew it was unheard of for the thesis mentor not to coauthor their student's work. I immediately began to have doubts. I asked him whether the work was unworthy of his joining as an author. He said something to the effect that it was more a compliment than a diss: He really liked what I had done, but he had played very little role.

I remembered then how I had held my tongue earlier when he chose not to include me on his first solo paper. I saw his action as both generous and, perhaps, a form of payback for the earlier omission (although we never discussed this), and also as evidence of his enormous integrity. This view of his integrity has remained the cornerstone of my deep appreciation of my thesis mentor.

I wrote the paper, probably with a great deal of help from Dale, but with me as the only author, and submitted it before Christmas break in 1976. A few days later, I was back East in Massachusetts, visiting my

parents. Dale called to say that the paper was returned with a statement from *Nature* saying that it was possibly interesting but belonged in a more specialized journal. Crestfallen and having never, at least back then, heard of a paper being returned unreviewed, I was quite unsure of myself and my future.

When I got back to Saint Louis, I set to work on a longer version for the *Journal of Physiology*, which at that time was the journal of record for cellular and electrophysiological neuroscience. That spring, Dale was invited to a Dahlem Conference in Germany and presented on my work on the submandibular ganglion. A few weeks later, Miranda Robertson, *Nature*'s leading biology editor, described the meeting in *Nature* (Robertson 1977) and wrote:

> Investigation of this phenomenon [of synapse withdrawal] in nerve-nerve synapses has been made possible for the first time by the discovery of J. Lichtman, reported by D. Purves (Washington University), that four out of five terminals made by guinea pig submandibular ganglion cells are withdrawn within four weeks after birth. The broader implications of selective synaptic elimination are suggested by the proposal (D. Hubel, Harvard Medical School) that the period over which it occurs may correspond to the 'critical' period within which the development of the central nervous system can be influenced by its input.

Aside from the report mistaking guinea pigs for rats, I was buoyed by the fact that my mentor chose to talk about my work, and by the sweet irony that it was interesting enough for an esteemed *Nature* editor to write about, but perhaps not interesting enough for the same editors to send for review just months earlier. I suspected that a sole-author paper by an unknown graduate student from Missouri probably never had a chance.

Robertson's piece was published one week before I submitted this work to the *Journal of Physiology*. I had two excellent scientists as reviewers (Phillipe Ascher and Constantino Sotelo—years later, each admitted to reviewing my first paper), and the paper was out in December 1977 (Lichtman 1977). Concurrently, several Italian neuroscientists were making the case that the one powerful climbing fiber that drives the activity of mature Purkinje cells in the cerebellum is also preceded in early postnatal life by a period of multiple climbing fiber innervation (Crepel, Mariani, and Delhaye-Bouchaud 1976; Delhaye-Bouchaud, Crepel, and Mariani 1975). Hubel, Wiesel, and LeVay (1977) were showing that segregation of ocular dominance columns in the visual cortex also occurred as axon branches were pruned.

Dale and I then did a project that showed that synapse elimination was not restricted to cells that ended up with only one strong input because synapse elimination also occurred in SCG cells that ended up with multiple inputs, but had twice as many at birth (Lichtman and Purves 1980). It was becoming abundantly clear that neurons in mammals were connected differently in early life than later on and that the removal of connections was widespread. My second solo thesis paper delved into what I considered an important question about the underlying cellular mechanism driving this synaptic reorganization. This paper was also the beginning of the conceptual approach that has been my constant companion for 45 years.

This second study of the submandibular ganglion posed the dichotomy at the root of the mechanism of synapse elimination: Was this withdrawal related to axonal territoriality or was it related to the control of individual postsynaptic ganglion cells? To distinguish between these alternatives, I thought it would be useful to map the locations of all target cells innervated by each preganglionic axon that converged on a cluster of ganglion cells. I reasoned that if axons mutually inhibited one another from occupying the same territory, then contiguous target regions would each be innervated by a different axon in the adult. I thought that the cases in which an adult cell was innervated by more than one axon might be at the borderlands between axonal territories. If, however, each ganglion cell was innervated by one powerful axon, but each target cell was the extent of that axon's territory, then the target cells themselves might play a role in choosing which axon stays and which goes. To decide between these alternatives, I used sequential pair-wise intracellular recordings in a cluster of ganglion cells. For each pair, I ramped up the voltage applied to the lingual nerve's incoming preganglionic axons to determine whether they were activated synchronously (same axon) or sequentially (different axons). I then removed the electrode from one member of the pair and went on to record from another cell. If cells A and B were innervated by the same axon, and after moving the electrode from cell A to cell C, I could tell whether all three were innervated by the same axon.

By sticking cells more than once, this experiment gave an unambiguous answer: Each nerve cell was captured by a powerful axon, but neighboring ganglion cells that were immediately adjacent and sometimes touching often did not share any innervation. This result strongly implicated the postsynaptic cell in choosing which axon was allowed to add synapses while the other inputs to the same cell lost them. I published this paper, like the first thesis paper as sole author (Lichtman 1980).

The result of this work was impactful for me in two ways. First, it showed that synapse elimination was likely based on competition but indirectly through a judge. I imagined that each target ganglion cell listened to the activity of incoming synapses and then chose, much as female birds listen to male birdsong, to choose a mate. I thought that what made all the

synapses of one axon able to compete against the synapses of other axons was that they acted as a unit, because they were synchronously active and could bring the target cell to the action potential threshold. This activity-centric view of synaptic competition has remained a theme in my work, albeit with many nuances over the years. The second impact was that it demonstrated the power of tracing the synaptic connectivity of multiple axons within a single sample. When I was doing this work, it felt quite fresh and powerful to visualize the synaptic connections of many axons within a single sample. I now see this as my first foray into "connectomics" (long before this word existed), and in this instance, I used an all-physiology approach. But the motive behind so much of my work henceforward was to do this same experiment, but at ever larger scales and in less accessible parts of the nervous system.

By the time I left Dale's lab to resume my clerkship clinical training in medical school (the doctorate was sandwiched between the first two years and the clinical year), I had coauthored 14 papers. This large number was thanks largely to Purves's impressive work ethic, and we had a lot to say! We wrote together several review and perspective pieces that suggested that, as a team, we had staked out an interesting point of view (Purves and Lichtman 1978, 1980; Lichtman and Purves 1983; Purves and Lichtman 1985a). My thesis defense was held on a Saturday. It was attended by my examiners—Max Cowan (see volume 4), chair of the Anatomy and Neurobiology Department; Cuy Hunt, chair of Physiology and Biophysics; Richard Bunge, a well-known neurobiologist interested in glial cells; Carl Rovainen, an electrophysiologist who studied the lamprey nervous system; and Victor Hamburger, the great developmental biologist—Dale Purves, and me. As I remember it, they didn't give me a particularly hard time, and the whole affair was over in a bit more than an hour. My experience was different from most modern theses, in which family and friends attend the public talk, followed by a private defense, and usually, a party for friends and family. For me, no audience, no friends, no party.

With the doctorate behind me, I had another important decision to make: what to do next. The clinical training for MSTPs lasted only one year because the fourth year was reserved for electives, and the doctorate was considered more than enough elective time. So, as I began the third-year clerkship in each clinical area, I had to decide whether to do an internship. Most MSTPs went on to complete an internship. I was already pretty sure this would not be the path I would take. I had a lot of momentum in my research and plenty I wanted to do. So, I entered the clerkship year hoping it would go quickly.

To my surprise, I liked clinical medicine a great deal. I liked talking to patients, delivering babies, and even sitting at the bedside of the dying during their last agonal breaths. Everything about birth and death, suffering and surviving, and the role of the physician in a person's life history

completely fascinated me. I had a newfound respect for what "life" was. As a graduate student, I had sacrificed many laboratory rodents and became inured to the slaughter taking place by my own hands. But the momentous firsthand experiences of delivery and death in the clinic changed my perspective and resensitized me to the miracle that life was.

I had a crisis on my hands: Should I give up such a rich career focused on saving lives for the more selfish pursuit of diving deep into something as esoteric as synapse elimination in the rat submandibular ganglion? My mother was sure that it made no sense to focus on rat spit when I could be a doctor. My father was less adamant, but I remember phrases he used like "not burning bridges" to argue that if I missed out on clinical training, there was no going back. In the end, however, I decided that while research and clinical medicine were equally challenging, I didn't have the stamina or intellectual skill to do both—as was expected of those have combined medical and philosophy doctorates—and so I decided to go with what seemed to me the easier path, continue with research, and give up any pretense of being a physician.

Choosing a postdoc lab was also fraught. Purves thought that I needed to go to the epicenter of brain studies: the Neurobiology Department at Harvard Medical School (HMS). I thought David Hubel (see volume 1), not yet a Nobel Prize winner, would be ideal, given his interest in pruning. Dale did not think this was a good idea and arranged for me to work with Paul Patterson, a terrific scientist, who was one of the first neurobiologists to take what we now call a molecular approach. I am sure I made both angry when I decided that molecular approaches were not of interest to me. I settled on the lab of Eric Frank, who was studying connectivity specificity in the bullfrog tadpole spinal cord.

I joined Eric's lab at HMS in 1980. Shortly after I got there in the summer of 1980, I met the beloved chair and founder of the Neurobiology Department, Stephen Kuffler, who, when he heard about my spinal cord project, seemed unimpressed by what I might learn and by whether this work was any different from studies being done in rats. Nonetheless, I really enjoyed working on this preparation that bridged the gap between the peripheral nervous system (PNS; muscle fibers, sensory spindles, and dorsal root ganglion) and their central projections in the spinal cord. I continued my work on studying single axonal arborizations and the basis of their specificity (Lichtman and Frank 1984; Lichtman, Jhaveri, and Frank 1984).

Tragedy occurred a few months later when Kuffler died unexpectedly on a weekend trip to his home in Woods Hole. The department was unusual in that it had a number of spectacular nontenured faculty who had no hope of tenure, because the six senior faculty—Kuffler, Hubel, Wiesel, Kravitz (see volume 4), Furshpan, and Potter—were the extent of the tenured faculty that HMS distributed to that department. The junior

faculty knew this, but also knew it was the best place in the world to be, and they expected that after their stint in Kuffler's department, they could get a tenured position elsewhere. That calculus changed with Kuffler's death—now there was an open slot.

Things grew more unstable a year later, in 1981, when Hubel and Wiesel were announced as Nobel Prize winners alongside Roger Sperry. Wiesel relatively quickly decided to leave HMS for Rockefeller and planned to take several junior faculty with him as well.

This was my first, but not last, experience of a department in turmoil. At a memorial service for Kuffler, I spoke with my former chair, Cuy Hunt, who had worked with Kuffler on muscle spindles. He asked me how things were going, and I said the department seemed unsettled, with morale slipping. I said offhandedly that I yearned for the tranquility of his (my former) department, where nothing seemed to get in the way of work. He said he would be happy to have me come back as a faculty member. And on a handshake, we settled that I would return to Saint Louis when ready. So, in the spring of 1982, I went back to Saint Louis, officially as Cuy's postdoc, to begin an assistant professorship in 1983. I didn't look for jobs, and as far as I know, there was no advertisement for the job I got. I also did not negotiate a salary or a start-up package. I did, however, write a grant to the NIH for a Research Career Development Award that paid my salary (a measly $40,000) for five years. So, I was on the tenure track and my own boss.

My second stint in Saint Louis lasted from 1982 to 2004. I began as a senior postdoc with my own lab space under the official mentorship of Cuy Hunt, who was still the chair of the Physiology and Biophysics Department. Cuy was an exceptionally nice and astute person. He was not a gifted teacher and spoke quite haltingly in the classroom. He also rarely convened faculty meetings; instead, he would intermittently show up to inquire or inform. He had a deft touch. His department was quite special; it was home to faculty representing physiology broadly. There was expertise in lungs, kidneys, muscle, cellular neuroscience, systems neuroscience, cell physiology, and ultrastructure of cells, among other areas. I benefited enormously from this diversity.

Cuy provided me with several pieces of advice that helped me in dramatic ways. The first advice he gave was to consider a new preparation. When I came back to Saint Louis, I thought I would restart my work on the submandibular ganglion. Cuy understood the advantages of a highly accessible preparation and suggested one that was even more accessible, but entirely novel: the snake transversus abdominis muscle. Cuy had published a couple of papers on the structure and function of the costocutaneous muscles (Hunt and Wylie 1970; Fukami and Hunt 1970), and he told me he found that another thin snake muscle is present as a left-right pair in each spinal cord segment (and there were hundreds of segments in each snake).

The idea of working on a cold-blooded reptile and working on muscle (my graduate work was on neurons, and my postdoc was on spinal cord) seemed a step backward, especially because the neurobiology of human specialness was where I thought I was headed. But I was intrigued enough to get a common garden garter snake and take a look. The transversus abdominis was only one muscle fiber thick, so it shared the excellent optical access that I enjoyed in the submandibular ganglion. But unlike the submandibular ganglion, the entire connectional pattern was fully in view and more stereotyped in structure. This accessibility enabled many additional types of analysis and a fuller realization of the approaches I was unknowingly beginning to develop in connectomics.

Just looking at the snake muscle under the microscope, it was immediately apparent to me that the muscle fibers were not all the same diameter, implying more than one type of muscle fiber. More interesting, they seemed to alternate: Every other muscle fiber was small. As I was trying to see the synapses on these two types of muscle fibers, I had the idea to take advantage of a remarkable discovery made by John Heuser and Tom Reese: that synaptic vesicle membrane was "recycled" after vesicle fusion with the presynaptic terminal membrane (Heuser and Reese 1973). They showed that the marker enzyme HRP could be internalized by a synaptic terminal following repeated synaptic activation. I wondered if I could do the same with fluorescent dyes. After a few failures, I asked Phil Stahl for advice. He was a faculty member in the department (who later replaced Cuy as chair and changed the department's name from Physiology and Biophysics to Cell Biology and Physiology in 1984). I sought his advice because of his expertise on vesicles and membrane trafficking. He suggested I try acidic fluorophores. I took his advice, and it worked! I found three fluorescent probes that are selectively internalized by active nerve terminals; they are sulphonic acid derivatives of common low-molecular-mass fluorescent molecules (rhodamine, fluorescein, and 8-hydroxypyrene). Because each probe emits at a different peak wavelength (red, green, and blue, respectively), I found that sequential stimulation of individual motor axons in the presence of different-colored probes allowed visualization of the relationship among terminals of different axons (Lichtman, Wilkinson, and Rich 1985).

In using this approach, I saw several startling things I hadn't been looking for. First, the thinner muscle fibers had multiple neuromuscular junctions distributed at regular intervals along their length, whereas the thicker fibers each had one neuromuscular junction. Second, the thin fibers almost always had more than one axon at each neuromuscular junction, as seen by the multiple colors of synaptic boutons at each neuromuscular junction. In contrast, the thicker fibers were all singly innervated because all of the synaptic boutons at a junction were of the same color.

Using sharp electrodes, my first postdoctoral collaborator, Bob Wilkinson, who ultimately became a professor in this department, and my first graduate student, Mark Rich, an MSTP, found that the small muscle fibers could not twitch because they lacked voltage-gated sodium channels needed for action potentials. In contrast, the large muscle fibers were of the twitch type and propagated action potentials along their length. Because a "tonic" muscle fiber could not propagate action potentials, the only way such a fiber could contract along its length was by distributing multiple neuromuscular junctions along its length to locally depolarize and hence contract the muscle fiber at many sites. But most interestingly, the fluorescence staining showed they also maintained multiple innervations at each of these distributed junction sites.

It took four decades, but my lab colleagues and I published a paper in 2026 that finally explains why both the single neuromuscular junction and its single innervation require postsynaptic action potentials (Meirovitch et al. 2026). Back to the past, Bob Wilkinson and I followed up with an even more complete connectomic analysis of this muscle, including full maps of the distribution of axons, synapses, and the realization that two types of twitch muscle fibers also alternated (Wilkinson and Lichtman 1985; Lichtman and Wilkinson 1987).

One major change during this period was that Dale Purves, Josh Sanes, and I switched departments in 1985. At the time, Cuy Hunt was retiring as chair, and Phil Stahl was moving the department more toward cell biology; an agreement was made between Phil and Gerry (Gerald D.) Fischbach (see volume 9), who was chair of the Department of Anatomy and Neurobiology at Washington University School of Medicine from 1981 to 1990, for the three of us to move across the hall to Gerry's department. I don't remember much discussion about this or even whether my opinion was sought. This switch put us among an outstanding set of neuroscientists—many of whom studied the brain with physiological and anatomical approaches. Even though I was a neurobiologist, up until this point (except for my brief excursion into the spinal cord with Eric Frank during my postdoc), my frame of reference had been cellular physiology and anatomy of the peripheral nervous system. So, this exposure was eye-opening.

Shortly after my return to Saint Louis, Dale embarked on perhaps the most important aspect of our continuing collaboration, recruiting me to help write a textbook, *Principles of Neural Development* (Purves and Lichtman 1985b). We had access to an empty room in the Cancer Research Building near our labs and spent the first two hours of every weekday morning for a year or more writing and revising the text. The book was arranged chronologically, beginning with neurogenesis and ending with the development of behavior. All told, there were 12 chapters, each speaking to the principles of the various steps in brain development. We wrote

the book in this order, and I remember many deep-diving conversations about how brains come into being.

Dale liked to argue about scientific matters, and so did I, so many ideas emerged during the writing that were probably not in either of our minds when we sat down on any particular morning. It is difficult to convey how joyous this effort was for me. When we were done, Sinauer published the book. It was well received, and the field of developmental neurobiology was in its heyday, so developmental neurology courses were popping up everywhere, and our book fit the bill. We wrote this book just before the advent of molecular neuroscience. Its perspective was more in tune with a cellular accounting of brain ontogeny than with either a systems-biology or a molecular approach. For better or worse, this cellular perspective has remained the cornerstone of my worldview on brain organization and its origins. Indeed, when Andy Sinauer, the publisher, said a few years later that it was time for a second edition, we were in a quandary. Molecular neurobiology was now in full swing. Many of the leading lights in development, such as Corey Goodman (see volume 12), Carla Shatz, Larry Katz, Mike Stryker (see volume 11), Tom Jessell, and our own departmental colleague Josh Sanes (see volume 12), were turning their attention to the roles of genes and proteins in neural circuit development.

For me, the challenge of writing about molecules was that there are so many of them, and each molecular storyline is related to its unique evolutionary trajectory. There may not be easily described, top-down, overarching principles beyond the power of natural selection to find solutions that improve fitness over phylogenetic time. I argued that taking the 12 chapters on cellular principles and adopting a more molecular view would require a chapter for each molecular pathway, and that would number in the hundreds, if not more. Furthermore, in most cases, the actual molecules were not known in detail; we only knew their names and, sometimes, their locations. But we had no structural biology to explain exactly how these molecules worked, what their structures were, and how they interacted, and with whom. This level of abstraction made molecules hard to discuss in the kind of fundamental way we could explain cellular principles. We never wrote a second edition.

At this time, a new technical and theoretical hurdle emerged in the research Dale and I were doing, related to inferring dynamic changes when the only data we had were static images from different time points across different animals. What was needed were ways to revisit the same neuron or synapse multiple times over days, weeks, or months. In 1986, Dale and his postdoctoral colleagues showed it was possible to image the same neuron's dendritic arbor in the SCG by injecting the same neuron with a fluorescent dye weeks or months apart (Purves, Hadley, and Voyvodic 1986). This seemed like an amazing result to me. But to study synaptic plasticity, labeling nerve terminals was required. Given my interest in

synapse elimination, it seemed like finding dyes to vitally stain neuromuscular junctions would be the best way to get started.

Dale and I tried methylene blue to study reinnervation of synaptic sites on autonomic ganglion cells, but it wasn't ideal for long-term study of the same synapses because it appeared to be toxic (Purves and Lichtman 1987). After Lorenzo Magrassi, a neurosurgeon from Pavia, Italy, joined Dale's lab as a postdoc, the three of us took on the challenging task of finding a good fluorescence probe that could be applied to living mice and stain synapses without toxicity; 18 dyes of a class that show affinity for mitochondria were tested at the mouse neuromuscular junction because of the high density of mitochondria in neuromuscular synapses. Several dyes of the styryl pyridinium family turned out to be remarkably good stains for motor nerve terminals; the best of these probes4-(4-diethylaminostyryl)-N-methylpyridinium iodide (4-Di-2- ASP) appeared to be nontoxic, highly specific for neuromuscular junctions in various species, resistant to bleaching, inexpensive, and easy to use (Magrassi, Purves, and Lichtman 1987). In an accompanying paper and subsequent work, we showed that the same neuromuscular junction could be studied over the life of an animal (Lichtman, Magrassi, and Purves 1987; Balice-Gordon and Lichtman 1990). The result was somewhat against expectations because junctions were quite stable; while they enlarged with the animal's growth, this was mostly due to growth like a hand, getting bigger without adding or remodeling branches.

A few weeks before we submitted this work on time-lapse imaging of synapses, my second child was born. Linda and I were already parents to Flora, who now hosts *Science Friday*, the public radio show and podcast. David was born in late May 1987. He was a beautiful child. About seven months later, he began to show dark bruises, and after an extensive workup at the Children's Hospital at Washington University School of Medicine, a few buildings away from my lab, he was diagnosed with neuroblastoma. I learned that this cancerous sympathetic ganglion cell precursor invades the bone marrow, crowding out blood-forming cells, reducing platelet counts, and impairing blood clotting. We felt we were in good hands; the oncologist assigned to us was an expert in neuroblastoma, and he had identified MYCN (n-Myc) as the leading proto-oncogene associated with the disease. He has gone on to have a distinguished career. Also reassuring was that he seemed to know me through the *Principles* textbook Dale and I wrote, and we could chat easily. Neuroblastoma is often treatable in children. We were given reason to hope despite the devastating news. I was also struck by how closely this work aligned with my area of study and how fortunate I felt to have an attending physician, given his expertise in autonomic neuron-derived malignancies, who was easy to talk to. After making the diagnosis, a chemotherapy regimen was initiated, and we were sent home. The first round of chemo did not bring the platelet

number back up, and so the disease was not yet in remission. Over the next five months, Linda, David, and I were in and out of the hospital as David received progressively more intense chemotherapy regimens, many platelet transfusions from anonymous donors, and multiple treatments for the complications of the profound immunosuppression of chemotherapy. None of these regimens brought a remission. Our respective siblings and my wife's mother came to stay in two-week stints to take care of Flora, as David's hospitalizations became more frequent and longer. When the stays were especially difficult, my wife and I would take turns in the hospital so we could get some sleep. After more chemotherapy sessions than I can count, we began to realize nothing was working. My father was a hematologist, and I spoke to him by phone at length. He was unsure of the diagnosis, but he was not an expert on neuroblastoma. At some point that year, he had a myocardial infarction that neither he nor my siblings told us about to spare us the added grief. But we began to have serious doubts that we were on the right path, and we expressed our frustrations with the nursing staff and residents who became highly familiar given our frequent hospital stays. Ultimately, these discussions were deemed provocative and led to a meeting in which the chair of the Pediatrics Department and our attending oncologist took my wife and me to an office to discuss the case. I remember they were both wearing newly starched and pressed long white coats with their names and hospital insignia embossed in red. The contrast between their pristine appearance and the grubbiness and blood that were at our son's bedside day in and day out was striking. They were stern. We were told that they were in charge and everything was under control. They insisted that we needed to trust them and that our comments were destabilizing and upsetting to the staff. We didn't argue, and I think we tried to comply. A few weeks later, we were summoned for a second meeting. This time, they gathered the larger hematology-oncology faculty. They told us that on further analysis with additional antibody screens, they were now convinced that David did not have neuroblastoma but a much rarer malignancy, acute megakaryoblastic leukemia. This disease required different and more intense chemotherapy, and I surmised it had a far worse prognosis. David never did go into remission and died in June 1988, 27 days into the second year of his life. It was a traumatic death, with my wife and me in attendance until we were ushered out of the room at a point when David appeared to me to have had a cerebrovascular bleed.

If there are lessons to take from such a tragedy, they are mostly lost on me. I have wondered if the delay in the correct diagnosis allowed the malignancy to be too large to treat, or whether the inappropriately targeted early chemotherapy allowed time for selection of resistant clones. But after David died, I lost all curiosity about his disease. I never understood why this happened and have tried not to dwell on what, if anything, could have altered the outcome. I have, however, had a lot of time to consider,

in relation to my work, the enormous challenges an expert clinician faces. A human brain becomes increasingly tuned to a worldview, a perspective shaped by the particular experiences it encounters. All the thinking we do as researchers or clinicians is subject to the biases that we bring to bear, based on what we know. This filtering leads us down our individual life paths and forces us to make decisions consistent with them, for better or ill.

Linda and I had to turn our attention to the living. We worked very hard to maintain our marriage for the sake of our lovely and wonderful daughter, Flora. Three years later, in 1990, Ruth was born. She has become an artist, a filmmaker, a director, and, like her sister, has given us so much to be thankful for.

The profound sadness of mourning was something my wife Linda and I dealt with in a similar way; we dove deep into our professional work. Linda was a journalist and worked at all three of the main publications in Saint Louis, ending our time in Saint Louis as a columnist for the *Saint Louis Post-Dispatch*. She was a celebrity. She seemed to know everyone in our home city and hobnobbed with the city's political and business elites. I was engrossed in my research. With my first graduate student, Mark Rich, an MSTP, we studied synapse elimination in muscle using time-lapse approaches made possible by 4-Di-2-ASP (Rich and Lichtman 1989a). The time-lapse approach also gave Rita Balice-Gordon and me deep insight into the role of activity in synapse elimination, as I will describe (Balice-Gordon and Lichtman 1994) and several other subjects where the time dimension gave new insight into synapse formation, synapse elimination, and maintenance, even over more than a year (Rich and Lichtman 1989b; Rich, Colman, and Lichtman 1994; Balice-Gordon et al. 1990; Balice-Gordon and Lichtman 1990, 1993).

In 1988, I also continued to pursue activity-dependent uptake of fluorescent dyes to label synapses and began combining this approach with the 4-Di-2ASP approach in hopes of visualizing the sites of vesicle recycling and mitochondria within the same synapse. But I was stymied by the scatter of the two dyes' fluorescence signal. I noticed that if I closed the field aperture on the exciting light to only illuminate a small spot on the specimen, the contrast in the small spot was far better than when the aperture was open, and I could clearly see the location of the mitochondria and the location of the recycled vesicles in individual boutons. I thought that if I could scan the preparation with a spot of light, then I should get a far better image. So, I set about making a "flying spot" microscope, although at the time I had never heard of a flying spot (Young and Roberts 1951). As I was building a rotating aperture in the field plane, Cuy Hunt dropped in for a chat, as was his style. He asked what I was up to, and I explained what I was trying to do and described the steric challenges related to the small area I had to work with for the rotating aperture. He made a simple suggestion: Why not put the flying spot along the microscope's vertical axis, perhaps behind the objective? I knew that the back focal plane of the objective

would not generate a spot (it is the aperture plane, not the field plane), but I wondered what would happen if I put the spot aperture in the image plane of a low-power objective and then used that plane as the field plane for a second high-power objective? In this way, the excitation light would be focused on a spot in the specimen plane, and the return emission light that went through the high-power objective below would pass through the same spot on its way back to the eyepiece of the camera.

I put this together in a few days using a Dremel tool to rotate a black piece of paper with holes punched by a sewing machine. When I turned on the rotating disk fast enough that I could no longer see the spots whizzing by, I had trouble believing my eyes. Although the pinhole density was uneven, causing the rotating disk to produce a streaky image, I could clearly see the two fluorophores with no scatter. But the image was even better, because as I focused up and down, the out-of-focus light disappeared. The image was sharper than the widefield image, had greater contrast, and minimized out-of-focus light. I knew I was on to something, and when I showed it around, some of my colleagues suggested I talk to the Washington University Office of Technical Development to apply for a patent. It was during my search for "prior art" that I rediscovered an idea developed by Marvin Minsky (albeit along a different optical path), based on work he did as a Harvard Junior Fellow. His idea was memorialized, not in a scientific publication, but through a relative, a patent attorney, who helped him secure a patent. Minsky described the first of what was later known as confocal microscopy. (Minsky 1957, 1988). Despite the patent, he never received any compensation for inventing confocal microscopy. His goal was to use confocal optical sectioning to disambiguate cells in Golgi-stained brain samples, presumably to obtain the wiring diagram. He was way ahead of his time. Although Minsky was unquestionably a genius, after I invited him to a summer course at Cold Spring Harbor to describe the history of this great invention, I realized he was not a gifted verbal communicator. Indeed, I think the inability to communicate how this approach could be used to reconstruct the wiring of brain cells may have spurred him toward the early ideas of "artificial intelligence," which became his area for the rest of his illustrious career at MIT.

The problem with Minsky's design was that it required two pinholes: one in the illumination pathway and one in the detection pathway. Once he aligned them onto the same spot, the only way to scan the specimen was to move it rapidly in the X and Y directions. He did this with two electromagnetic tuning forks. It was very clever but impractical for squishy biology samples, which would not tolerate being shaken by the oscillating stage. The way I was doing the scanning, the only thing that had to move was the spots, which served both the incoming and outgoing light (hence a dual aperture). I soon realized I could use a slit to get very fast confocal images in all directions except in the narrow axis of the slit.

I wrote a number of patents (e.g., see Lichtman and Sunderland, 1989) and published a paper (Lichtman, Sunderland, and Wilkinson 1989) describing this approach. I made a mistake by publishing the work in the inaugural issue of *The New Biologist*, which I had been invited to found along with several more distinguished colleagues. The cover of this first issue showed the superimposition of 4-di-2Asp and activity-dependent vesicle labeling of a snake synapse via my confocal microscope. The journal, however, survived for only a year. Nonetheless, there was interest in the technique. I was named "Missouri Inventor of the Year," had a full-page write-up in the *Post Dispatch*, and Newport Corporation licensed the technology to make a spinning disk confocal based on my design. The royalty payment was enough to trade in a Ford Escort with 100,000 miles and buy a brand-new Mazda Miata the first year it was sold in the United States. Since then, I've participated in more than 25 patents, but none have given me anywhere as much financial reward as this first one, a true example of beginner's luck. A Japanese company later modified the design to add a second spinning disc with microlenses, illuminating the holes in the spinning disc more brightly. That spinning-disk confocal, 35 years later, is still a standard part of the confocal armamentarium, and although I receive no financial reward, I feel some pride that its dual-aperture design is based on my earlier work. After this, I dabbled in developing other new kinds of light-based microscopes, usually with the goal of answering a question related to synapse structure or development (e.g., see Conchello et al. 1997; Lu, Min, et al. 2009; Marques, Conchello, and Lichtman 2000).

My lab became increasingly focused on the mechanism of synapse elimination. It was now the 1990s, and I was a tenured professor. Because I was at a medical school, I had only minimal teaching responsibilities, and plenty of funding, so I could devote myself fully to the mechanisms behind synapse elimination, the subject I began as a graduate student more than a decade earlier. I insisted on taking a cellular approach, even as many of my contemporaries veered strongly into the molecular domain. Gerry Fischbach, my chair, at some point during an annual review, said, in effect, "Surely, sooner or later, you will be headed toward the molecular mechanism of synapse elimination." I was less sure but told him that once I had the cellular details worked out, I would presumably go in that direction. But then, as now, I continued to find fundamental, unanswered questions that seemed worth studying at the cellular level.

This stubbornness on my part got me embroiled in many arguments, especially with Josh Sanes, my faculty colleague and, eventually, my long-term collaborator, who had built an enormously successful career dissecting the development and organization of the nervous system using molecular approaches. It was hard to argue with his success despite its "molecular-centric" direction. We found common ground on the pitfalls of molecular analyses, however, in our view of an area neither of us studied: long-term

potentiation (LTP). We wrote a piece with the title "Can Molecules Explain Long Term Potentiation?" that earned both of us a reputation for contrariness but accurately represented the challenges of transforming a cellular phenomenon into a molecular explanation (Sanes and Lichtman 1999; Lisman, Lichtman, and Sanes 2003). Although I could argue about the supposed virtues of molecular explanations, there was no doubt that molecular approaches could create tools that completely changed the way my lab did its work. Josh was responsible for many of these developments, and more than 30 collaborative papers with my lab show the immense power of his molecular approaches to tool development.

Our initial time-lapse studies of neuromuscular synapse elimination indicated that it was associated not only with synapse loss but also, at least sometimes, with the postsynaptic sites they occupied. This result argued that whatever was driving synapse elimination was not just a shoving match between axons vying to occupy the same sites. My lab colleagues and I turned our attention to the choreography of the loss. The central question was what was the involvement of the postsynaptic cell in axonal elimination. We approached this question in several ways. First, we wanted a more quantitative way to assess acetylcholine receptor (AChR) density in the muscle postsynaptic membrane, which served as our marker of postsynaptic site loss. We developed a nonbleaching fluorescent standard that enabled a quantitative assessment of receptor loss (Turney, Culican, and Lichtman 1996; Akaaboune et al. 1999; Akaaboune et al. 2002). Second, we used physiological methods to show that the sites where receptors were lost were at least temporarily overlain by synapses capable of releasing neurotransmitters (Colman, Nabekura, and Lichtman 1997). Third, to track the fate of the lost receptors, we used focal puffing of fluorescently labeled alpha-bungarotoxin, a nearly irreversible ligand of the AChR, over small regions of a neuromuscular junction to determine whether they were fixed or could migrate. The result of this experiment was a surprise: The local receptor sites that were blocked with alpha bungarotoxin disappeared, and the overlying nerve terminal also retracted (Balice-Gordon and Lichtman 1994). This suggested that silenced local sites disappeared. However, when we silenced the entire neuromuscular junction, nothing happened. This implied that the junction's active region destabilized inactive sites. Charles Jennings, an editor at *Nature*, wrote a "News and Views" implying that the active sites were sending punishment signals (he used a red arrow in the diagram) and that the active regions were protected from this punishment signal (he signified this protection as a blue cloud) (Jennings 1994). This terminology permeated all my talks and many of my papers for about a decade.

The other theme that was becoming increasingly important was a deeper exploration of color as a means of distinguishing different axons, especially to better describe synapse elimination. Many aspects

of this competitive process of synapse elimination would be clearer if one could see both the eventual winners and losers as separate entities. Lipophilic dye labeling of individual axons was one strategy, as was activity-dependent labeling of different axons competing at the same junction (Balice-Gordon et al. 1993; Gan and Lichtman 1998). It occurred to me that the number of available colors could be greatly increased by using a combinatorial approach in which each label was a mixture of several colors. Our first attempt at this was with lipophilic carbocyanine dyes such as 3,3′-dioctadecyloxacarbocyanine perchlorate ("DiO"), 1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate ("DiI"), and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine perchlorate ("DiD") . By using a "gene gun" to ballistically shoot small metal beads with various mixtures of these three dyes (DiOlistics), we could get a rainbow of different colors in a brain sample, and every axon was a different color (Gan et al. 2000). The full embodiment of this approach had to wait for another technological advance: genetically encoded fluorescent proteins.

Josh Sanes and I teamed up on a project that yielded an approach that became the mainstay of my efforts to understand synapse elimination from that point onward. The use of fluorescent proteins, notably green fluorescent protein (GFP), was just in its infancy in the 1990s, but I already knew it would be the ideal probe for longitudinal (time-lapse) studies of synapses, with the added bonus that, if it could be expressed in the cytoplasm of a neuron, one would be able to see the synapses and the axon. Josh enlisted Guoping Feng, a highly talented molecular biologist who was then a postdoc with Josh, to design a means for the genetic expression of fluorescent proteins. After considerable tinkering, the technique worked remarkably well and had one unexpected outcome: Many of the animals had heritable expression of the fluorescent protein (red, green, or blue) in distinct subsets of neurons (Feng et al. 2000). These lines are still in wide use more than two decades later. This approach opened up a wide range of experiments previously unimaginable. We studied the pattern of axon branch pruning in individual motor units (Keller-Peck et al. 2001), time-lapse analysis of synapse elimination (Walsh and Lichtman 2003), and the fate of retracting axons (Bishop et al. 2004). This latter study was also our first foray into serial section electron microscopy, which I felt was a close kin to confocal, just with much better resolution. We used it to study the ultrastructure of retracting axons being eliminated at the developing neuromuscular junction. We used the serial electron microscopy in combination with confocal fluorescent protein imaging for a correlative light and electron microscopy approach. This combo became a standard tool in my lab's work going forward.

During the decade between 1990 and 2000, I traveled weekly to have lunch with Victor Hamburger (see volume 1) at his home in University City. Victor, who died in 2001, was an emeritus professor in the Biology

Department, across the park from the Medical School. He was trained by the experimental biologist Hans Spemann, whose work with Hilde Mangold on "the organizer" earned him a Nobel Prize in 1935. In his own lab as an émigré from antisemitism in Europe, Victor supported both Rita Levi-Montalcini and Stanley Cohen, with whom he conducted key early experiments that ultimately led to their Nobel Prizes for nerve growth factor (NGF) and epidermal growth factor (EGF), 51 years later (1986).

One could easily argue that Victor deserved a share of the credit for the 1986 Nobel Prize, as my colleagues Josh and Dale argued (Purves and Sanes 1987). I have always had a somewhat dim view of Nobel Prizes on first principles, because of their arbitrariness, with no more than three awarded on a subject in a year, which discourages collaborative efforts, and because the awardee must be living to get the award, discouraging respect for researchers who were truly ahead of their time. In addition, Victor, who lived alone and was somewhat retiring, insisted that his longevity—he died at 101—would not have continued had he received the prize in 1986 when he was 86. I enjoyed these lunches. We covered a lot of ground over the decade, and I felt fortunate that someone 50 years my senior with so much lucidity, knowledge, and wisdom—spanning a century of experience—was willing to spend an hour with me each week.

For me, a particularly noteworthy series of discussions centered on comparing descriptive and experimental approaches to biological questions. Victor was trained by the preeminent experimental embryologist of his day, and much of his work continued in this tradition of transplantation, extirpation, and other manipulations. The work of Levi-Montalcini was equally experimental, based on studying the effects of adding exogenous chemical factors from tumors and glands to brain cells in culture, ultimately leading to the purification of NGF.

It was hard to argue that this was not a great example of the power of experimentation to answer fundamental biological questions. But I did (and still do) argue this point. Because much of my graduate and postdoctoral research, and my lab's research, was experiment free, it relied on description rather than experimentation. It depended more on discovery through observation than on hypothesis testing. I saw a clear disadvantage in using deductive experimental approaches, which are commonplace in biology, compared with inductive descriptive approaches, which were, frankly, frowned upon, for getting an answer. My argument was that an experiment requires a hypothesis and that most papers aim to show that this hypothesis is likely true. I insisted that this perspective almost always leads to trouble because the linguistic framing of a hypothesis is naïve compared with the phenomenon's actual hypercomplexity.

Victor mostly disagreed but was eager to debate the point. I was especially grateful that he introduced me to the work of someone he thought might be a kindred spirit, Walther Vogt (1888–1941). Vogt did fate

mapping with vital dyes to follow embryonic development in cold-blooded vertebrates. He was also willing to argue, even against the great Hans Spemann, that there are fewer interpretive difficulties in observing development than in manipulating it. This was laid out in a paper Vogt wrote in German, later reworked into a masterful book Victor wrote (Hamburger 1988), which contrasted the approaches of Spemann and Vogt. For better or worse, I have continued this tradition of taking my lead from the material I am studying rather than from my ideas. It was heartening to know that Victor believed the same thing. When discussing his experimental work on chick development, according to Johannes Holtfreter (1901–1992), he said: "Our real teacher always has been and still is the embryo—who is incidentally the only teacher who is always right" (Locke 1969).

In the early 2000s, I began to see the writing on the wall for basic research at a medical school. I loved Washington University, but I suspected that this institution was beginning to take its medical mandate seriously. The clinical departments were expanding their research faculty and new buildings were going up, but I felt that the potential for growth for "basic" research seemed stymied. I was offered a few promising jobs in coastal universities that were not in medical schools. I thought a move to a biology department might make it easier to justify the rather nonclinical direction most of my work was headed.

In 2004, I moved to Harvard's Department of Molecular and Cellular Biology (MCB) across the river from the medical school. Josh Sanes was also recruited at the same time, which was reassuring. At first, I was quite intimidated by the number of great and famous scientists. It didn't help that at the first MCB faculty meeting I attended, Matt Meselson, one of the fathers of molecular biology, pronounced that the quality of the new recruits joining the department was definitely "going down." But things steadied, and I found a friendly home among a sensational group of colleagues.

Although in a new lab, my work continued on the same path. From the transgenic fluorescent protein lines, it was just a short intellectual jump to making genetically encoded mice with many colors (i.e., "Brainbow" mice). Using the idea of combinatorial color mixing, as we had done with lipophilic dyes earlier (Gan et al. 2000), postdoc Jean Livet used the random recombination of multiple cassettes of red, green, and blue fluorescent proteins to generate mice that expressed multiple colors (Livet et al. 2007). In that paper, we coined the term "connectomic" in its current usage for the first time; now "connectomics" is legitimized in the *Oxford English Dictionary*. In 2008, when Tsien, Chalfie, and Shimomura won the Nobel Prize for fluorescent proteins, the *New York Times* used one of our Brainbow images in the central nervous system (CNS) on the front page to herald the prize. It was a thrill to see how quickly these fluorescent proteins became a standard part of the neurobiology armamentarium. We have used Brainbow to

great advantage to study synapse elimination in muscle (Meirovitch et al. 2026) and in autonomic ganglia (Tsuriel et al. 2015; Sheu et al. 2017).

Using Brainbow in the dense neuropil of the CNS proved far more challenging than in the periphery, where neurons and neuropil are much less densely packed. For example, using transgenic fluorescent protein expression with Ju Lu, my first graduate student at Harvard (who is now on the faculty at Lehigh University), we could even do complete analyses of the neuromuscular connectome of a small ear muscle's innervation (Lu, Tapia, et al. 2009). These complete maps were revealing. One thing we found was random variation in axonal branching, even between the left and right versions of the same bilaterally symmetric muscle. We have used this muscle in several subsequent studies (Meirovitch et al. 2026).

With the advent of expansion microscopy approaches, Brainbow-style fluorescence may ultimately be useful for CNS studies (Shen et al. 2020). Because of the challenges of the resolving power of fluorescence in the CNS, I decided, after our first serial electron microscopy study, to push ahead in that ultrastructural direction. I was fortunate to have two adventurous and highly skilled lab colleagues who had deep interest in this ultrastructural technical direction: Master electron microscopist Richard Schalek and Ken Hayworth (now leading the connectomics charge at Janelia Research Campus). We used scanning electron microscopy and a tape-based collection method thought up by Ken. We used this approach across a variety of species to reveal "saturated" reconstructions of the neuropil, showing that some excitatory axons establish multiple synapses on the same target cell in the mouse cortex (Kasthuri et al. 2015). We studied the development of connectivity in the nematode *C. elegans* (Witvliet et al. 2021; Mulcahy et al. 2022), the larval zebra fish (Hildebrand et al. 2017; Bahl and Engert 2020; Odstrcil et al. 2022), the marine mollusk Berghia (Sant et al. 2025), octopus (Bidel et al. 2023), and Hydra (Zhang et al. 2025). We assisted Zeiss in the development of a tool for connectomics, a multibeam scanning electron microscope (Eberle, Mikula, et al. 2015; Eberle, Schalek, et al. 2015). This had the same property as the spinning disc confocal. It used a multitude of beamlets to accomplish a scan faster than possible with a single beam.

In 2024, my lab colleagues and I partnered with Viren Jain's expert team at Google to reconstruct a petavoxel (~1,000 terabytes) of a small fragment of human temporal cortex removed from a patient undergoing surgery for epilepsy (Shapson-Coe et al. 2024). The entire volume was only one cubic millimeter. Along the way, we developed a suite of correlated light and electron microscopy tools (Fang et al. 2018; Han et al. 2023, 2024). We have also, thanks to the great skill and energy of postdoctoral associate Mariela Petkova, provided the electron microscopy data for a complete fish connectome in a collaboration with Janelia, Florian Engert's lab at Harvard, and Google. This will probably be the first

complete vertebrate connectome spanning sensory systems to muscle output. Because this same fish has also had its neuronal activity monitored using Ca++ indicator dyes, the relationship between circuit structure and function is direct.

From my career-long interest in how experience sculpts or otherwise modifies a mammalian nervous system, all this effort has been a prelude to whole-brain connectomics in adult and juvenile mammals. The NIH developed a program within the Brain Research through Advancing Innovative Neurotechnologies (BRAIN) initiative called CONNECTS to determine whether there is a path to obtain a whole-mammalian brain connectome. I am the principal investigator (PI) of one such grant that spans seven sites and has the goal of proving a proof of principle for getting the "mind of a mouse" (Abbott et al. 2020). This is an exciting venture that is enormously challenging from a technical standpoint and, ultimately, in regard to the possibility of making sense of a complete mammalian brain wiring diagram. The dataset, if we are allowed to continue, will be about 1 million terabytes. But that is a big "if."

My hesitancy about the future stems from a reality that hit us hard in 2025, when the government conducted a "mass termination" of all NIH and NSF funding to Harvard. Because of my PI role, Harvard was the home institution for this grant, and all my colleagues at other institutions, who were subcontractors, also lost their funding. I had the dubious distinction of having the most NIH grants in the Harvard Faculty of Arts and Sciences, so my lab was greatly affected in multiple unpleasant ways. The pretext for this termination was Harvard's antisemitism. Antisemitism was not obvious to me, at least in the Science Division of the Faculty of Arts and Sciences. It was not lost on me that the president of the Harvard Corporation, the president of the university, the provost of the university, and, at the time, the dean of science (me) were all Jewish. Harvard went to federal court and won the case, arguing that the action was illegal. As of this writing, the federal government announced it plans to file an appeal of the ruling. At the moment, the lost grants have been reinstated. There is no reason to feel this is the end of the federal efforts to curtail research at Harvard and perhaps other universities. I had taken for granted the US federal government's remarkably enlightened view of academic research. I was funded by a federal MSTP grant during my graduate work and had continual federal funding until that mass termination more than 50 years later.

Speaking of terminations, I took, and then lost, the job of dean of science in the Faculty of Arts and Sciences (FAS) at Harvard, 2024–2026. When asked to serve, I had, and still have, an active, well-funded lab and extensive teaching responsibilities, and I was no spring chicken at 74, so it was not obvious I would be a good choice. Although I was very busy, I felt I had reason to participate, even though I did not volunteer for the position.

When I was recruited to the job, I was hopeful that I would be able to do something that I felt needed immediate attention. The previous Science Division deans were faculty in the physical sciences, and I sensed serious neglect of the life sciences. With half of the division's departments having biology in their names, this was a serious problem. Given that the dean of the FAS and the president had a background in life sciences, I saw a great opportunity to build in this area. Alas, current events overtook this ambition with a double whammy: the mass terminations of federal grants and a growing unease relayed to me by upper management about profound budgetary shortfalls in the FAS. I was told that the situation required that we restrict faculty hiring, graduate student admissions, staffing, and the like. I was dismayed, given that Harvard was, and still is at this writing, the richest university in the world. I tried to do what I could to keep things moving toward my lodestar: the 10 science departments I served. But I suspect my view of leading from behind, which I made clear as a warning in my first interview for the job, was ultimately not what my bosses really wanted. Although I strongly felt my job was to convene the brain trust of faculty and faculty chairs to enable them to solve their problems, I think the administration sought a different kind of leader, more of a shepherd to guide the sheep. Basically, it was an issue of bottom-up vs. top-down leadership. My superiors felt that good leaders in middle management (such as a science dean) need to toe the line, to comply with the views of administrators in higher positions in the chain of command. This seemed more like following than leading to me. Not surprisingly, I was let go. Anyway, there was some brouhaha about my dismissal because the 10 departments I served seemed reasonably happy with my leadership approach. They were surprised and puzzled, especially when the official line describing my departure as "stepping down" rather than being fired, even though I had told all the department heads I was fired (Gerstein and Mahajan 2026; Thakkar 2026).

I was proud of what I accomplished in my short term of service—perhaps the shortest deanship in Harvard's history! I began a new educational initiative to introduce first-year students to genuinely hard problems (GHPs) and encouraged them to choose an unsolved scientific problem to work on throughout their undergraduate experience (Pattison 2025). A GHP is a problem in which, despite the best efforts of the extant scientific community, we have made little progress. These are areas in which the lack of conventional wisdom might be an advantage rather than a disadvantage. The goal is to have students see education in the sciences not as a prelude to confronting a hard problem, but rather as an ally, allowing the material learned to be relevant at the time of learning—not the standard long interval between school learning and science research. I also began a secondary field in entrepreneurship, with support from Harvard Business School, for our STEM students who are interested in science but may need

to look beyond the federal government for funding. I also changed how the Science Division office operated, including more faculty input than before, when the faculty dean worked with a team entirely of staff. I recruited three seasoned faculty members to serve with me on an executive committee, with pay, so that no decisions would be made without hearing multiple faculty viewpoints.

In any case, what happened to me may be symptomatic of something larger and more important. Research universities have outlasted corporations by hundreds of years (in the case of the Ivy League), but why? Unlike a company, an academic institution sees its value not in terms of profit but in the quality of its academic employees, the faculty. This is why administrative roles are usually filled by individuals with a faculty appointment. I am sensing, over the course of my time in academia, a gradual shift toward the creation of an administrative class that sees itself as a financial steward of the institution rather than as the humble servants of the academy of educators and researchers. New ideas almost always arise from the bottom, and if administrators care more about financial survival than the teaching and research mission, their institutions will perish, as most companies do, by ignoring the folks who know best what is going on in the trenches.

Afterword

Given that synapse elimination has been the central subject of my research for more than 40 years, it has colored my perspective on pretty much everything. One point that I have made repeatedly is that the widespread overconnectivity seen in the protracted developmental trajectory of human beings gives each of us nearly unlimited potential to become whatever our experiences lead us to be. In infancy, as we begin looking around the particular habitat in which we are born, as we begin listening to our parents speak in the particular languages they use, as we begin paying attention to the particular lessons our teachers and elders and eventually our cell phones are proselytizing, and as we begin the arduous struggle to master talking, walking, riding a bicycle, driving a car, and ultimately doing brain surgery or piloting a plane, we become truly expert at a small subset of what was initially possible.

At the same time, we become progressively narrower, less capable, or less interested in mastering other areas or seeing the world through a different perspective. Each of us is thus tuned based on our unique history. This tuning occurs through experience-based strengthening of some pathways, while our brains eliminate others. I suspect that all my strongly held views about science have been shaped by the power of experiences to remove from my cranium all but those few things that resonate with the few wires that remain. Although many of the ideas I believe are self-evident

to me, I realize that no one shares my experiences. It is not lost on me that the decades I have spent thinking about synapse elimination as the means to shaping the nervous system have likely shaped my brain to see this perspective as *the* explanation for who I am. Had my doctoral thesis been on growth factors like brain-derived neurotrophic factor (BDNF) or LTP, I might have been assertive about a very different perspective on how information is stored in the brain. In this sense, I consider myself lucky that synapse elimination left me with the right idea.

I should like to end this autobiographical sketch with what one might call my personal scientific philosophy, distilled from my life history.

My attempts to extract meaning from images have made me aware of the fundamental conflict between my responsibility as a scientist to explain my observations versus the innate complexity of what I am trying to lay bare. I believe that the process of explaining is so fraught that we should, as crazy as it sounds, sometimes, give it up entirely. Take the relationship between genotype and phenotype, or between the brain's wiring diagram and the behavior it gives rise to. I contend there is no way to explicitly describe the map from one to the other. This isn't because the former categories (genotype or connectome) are not causally related to the latter ones (phenotype or behavior)—they certainly are! But ferreting out the logic of those relations is stymied by the network properties of genomes and connectomes. We humans like to discretize things; after all, that is the purpose of language. But for highly interconnected networks, such as genes in a genome or neurons in a connectome, such linguistic partitioning downplays the essential requirement for interconnectedness. For me, language feels more like a hindrance than an advantage when dealing with the brain's evident hyperconnectivity. I see this playing out right now. As I mentioned earlier, my lab generated the dataset for the larval zebrafish, the first full synapse-level vertebrate connectome. The fully segmented and proofread wiring diagram was only possible due to a profoundly collaborative consortium with researchers at Google, Janelia Research Campus, and the lab of my departmental colleague Florian Engert. Although not yet published, many tens of labs are already deeply immersed in studying this wiring diagram. Those interested in the role of cerebellum can, with a mouse click, trace all the pathways of every neuron projecting to the cerebellum and every downstream pathway exiting this CNS hub. Similarly, visual system scientists can trace sensory pathways from retinal ganglion cell axons to motor action in the various extraocular muscles or swim musculature. But when I look at this network without names and labels, I am struck by the large number of connections that cannot be easily rationalized. When I follow a single primary sensory axon into the brain and trace its secondary postsynaptic partners, then the tertiary postsynaptic partners of those second-order neurons, and so on, I eventually label the whole brain. How do we use language to explain this?

This is not the only problem with explaining the brain. One might say that biology contains all the complexity it does because every bit of it is essential. In this sense, biological systems, such as the nervous system, can be considered algorithmically incompressible. Explanation, in contrast, by its very nature, attempts to compress complexity into more easily understood ideas, usually linguistic but sometimes mathematical. In physics, we have equations that powerfully encapsulate complexity (think the Schrödinger equation, the Dirac equation, or Navier-Stokes). Each of these is derivable from principles of physical law, and each yields testable predictions. But in biology, any evolutionary accident that occurred over the billions of years life has existed can become ingrained so long as it confers fitness on organisms that inherit the accidental change. These biological accidents arise from a genomic wellspring that is virtually infinite. This is why explaining, or fully understanding, the nervous system may be too lofty a goal. I know this sounds anti-intellectual, but decades of ruminating brought me to this perspective. To be clear, I don't see moving away from seeking a unified understanding or theory of the brain as defeatist, because there is another, richer avenue of inquiry available. Might we thus set our sights on a slightly more modest goal than understanding? Could we be content to use our unmatched technical prowess to describe the natural world as fully as possible? For neurobiologists, that would mean a full description of the brain. As I have recounted, "description" in neurobiology is often viewed dimly. I lived through an era when many of my papers were criticized by reviewers as "merely descriptive" because they lacked a clear hypothesis and an experimental manipulation to test it. I was undeterred. Eventually, I began to get reviews that contained phrases like: "despite being descriptive, something interesting was learned." Human genomes, connectomes, transcriptomes, dinosaur bones, or the cosmos-ome (sorry) add enormous insight not because they test a hypothesis but because they give rise to new ideas. Darwin's descriptions of bird beaks preceded his revolutionary ideas on natural selection. Katz's (see volume 1) observations of miniature endplate potentials preceded his quantal hypothesis of vesicular neurotransmitter release. Maybe describing neural networks in their entirety will bring forth regularities that can serve as evidence for those inclined to use their intelligence to formulate hypotheses.

Is our intelligence all that it is cracked up to be? Perhaps stemming from my insecurities about my smarts, or lack thereof, I have wondered generally about the potential limits of human brainpower. In the scientific community, there is an underlying, usually unstated assumption that nothing is beyond our reach; given enough time, humans are smart enough to figure everything out. No doubt humans have already figured out far more than any species before us. And as a neurobiologist, I see this remarkable ability deeply related to our brains. But I also know that our brains are only modestly different in their physical and chemical form from those of our nearest animal relatives. When we consider the behavioral repertoire of the

great apes in terms of what they have accomplished, they are more similar to rodents than to modern humans. As profound as our capacity for thought is, we cannot forget that this capacity is grounded in the same mechanisms found in other animal brains. Maybe it is just hubris, rooted in human supremacy over animals, that gives us this sense of unlimited capability to understand things. The dawn of the age of artificial intelligence only adds to our conviction that we (with the aid of machines) will figure it all out. For me, as a neurobiologist, the philosophical conundrum comes down to the challenge that the biological machine (i.e., the brain) that enables us to think is more complex than the thoughts it produces. So where does that leave us? Neurobiologists accept that the things we study hold secrets and insight we consider worth knowing. But we need to go one step further and admit that these systems are far more ingenious and subtle than human thought. To paraphrase Hamburger, we are the students, and the things we study are our teachers. Perhaps it will always be that way.

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