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Joshua Richard Sanes

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Assistant, Associate, Full and Alumni Endowed Professor, Washington University School of Medicine, St. Louis, MO (1980–2004)
Sabbatical, Institut Pasteur, Paris, France (1985–1986)
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Sabbatical, University of Marseille, Luminy, France (1999)
Jeff Tarr Professor of Molecular and Cellular Biology, Harvard (2004–present)
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Stephen Kuffler Lecture, Harvard Medical School (1996)
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Alden Spencer Award, Columbia University (2000)
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Visiting Professorship, College de France, Paris (2004)
Elected member, American Academy of Arts and Sciences (2006)
Grass Lecture, Society for Neuroscience (2008)
Bishop Lecture, Washington University (2010)
Javits Neuroscience Investigator Awards NIH (1989, 2005, and 2012)
Harvey Lecture, New York (2012)
Member, NIH Advisory Committee on BRAIN Initiative (2013–2014)
Vernon Mountcastle Lecture, Johns Hopkins University (2017)
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Joshua Sanes and his colleagues have studied the formation of synapses, which transmit information between nerve cells. For many years, they used the neuromuscular junction to identify intercellular signals that regulate formation and maturation of this model synapse. In the course of this work, they pioneered new ways to mark and manipulate neurons and the synapses they form. Subsequently, they turned to the retina to identify molecules underlying the specificity of synapse formation, which is responsible for assembling the complex circuits that underlie information processing. Most recently, they have used high-throughput transcriptomic methods to generate cell atlases of the retina in mice, humans, and other vertebrates and have used them to analyze the development, resilience, dysfunction, and evolution of retinal cell types.

Joshua Richard Sanes

Introduction

I have had a wonderful life so far: loving parents and grandparents, a long happy marriage, terrific children, good health, financial stability, the respect of my peers, and many friends in the neuro-verse. However, none of this would be of particular interest to the likely readers of this chapter. There have also been some bumps along the road, but they are likewise of modest general interest. So this will be a scientific autobiography—appropriate for the venue and appropriate to me, since my primary self-identification for more than a half century has been as a neurobiologist.

I view the core of my work as being about synaptic specificity—how axons choose particular targets (cellular specificity) and, in some cases, particular parts of a target cell (subcellular specificity) on which to form connections. My lab has undertaken three major efforts directed at this goal. The first used the neuromuscular junction (NMJ) to study subcellular specificity and, as the work unfolded, synaptogenesis in general. The second used the retina to study cellular specificity. The most recent applied high-throughput transcriptomic methods to retina to see if we could comprehensively classify and characterize the full cast of characters. The efforts overlapped, occupying roughly 1976–2010, 1995–2018, and 2014–present, respectively. Rather than taking a strictly chronological approach, I'll describe them in turn. To avoid abandoning chronology altogether, I'll intersperse these sections with ones on other projects and activities, and precede them with brief comments on my family and childhood.

Childhood

When I was a child, my parents seemed remarkably different from each other, but I now see astonishing similarities between them. First, both were children of immigrants from Eastern Europe—Russia/Poland (it kept switching back and forth) for my paternal grandfather, Romania for my paternal grandmother, and Lithuania for both maternal grandparents. Second, after my grandparents passed through several cities—Patten (Maine), Rochester (New York), Chicago, and Philadelphia—they ended up in Buffalo, where my parents grew up in a lower-middle-class predominantly Jewish neighborhood just a few blocks from each other. In fact, they were casual friends, although my father was three years older than my mother and they traveled in different social circles. Third, both of my parents had older sisters who died in childhood from conditions that would not be lethal today—Down's

syndrome and appendicitis. This left them both as only children in melancholy families. Fourth, both were very smart and—especially my father—fiercely intellectual. Fifth, both grew up with thwarted ambitions. Shortly after my mother, social and scholastic, enrolled in college as a premedical student, her father suffered financial reverses and she was forced to drop out. My father, too rebellious and politically active to finish high school, wanted to be writer, but was never able to make that his profession.

As a consequence, both ended up leaving Buffalo during World War II. My father was in the Army, serving as a radio operator in New Guinea. My mother moved to Utah with a friend, and then on to San Diego. There she worked in an airline control tower, bought a Piper Cub, learned to fly it, and volunteered in the Civil Air Patrol, flying up and down the coast looking for Japanese submarines. She didn't find any.

After the war, both returned to Buffalo, reunited, married, and moved to New York, where my father tried to write fiction, my mother worked as a copy editor for left-wing magazines, and both ran with a crowd of New York intellectuals many of whom remained lifelong friends. When my mom found out that she was pregnant with what became me, they moved back to Buffalo. My father joined his father in the latter's automotive parts store. In the optimistic postwar years, his idea was that he would soon make his fortune, leave daily operations to subordinates, and return to writing. Sadly, although he was able to support the family, the riches did not materialize. He remained at the store for many years, eventually became a salesperson for a college textbook company, and finally retired. At that point, he was finally happy and spent a decade making wonderful collages and enjoying his grandchildren (two of mine and two of my brother's) until he was taken by a glioblastoma in 1995.

My mother stayed home for around 15 years after I was born, volunteering in all manner of activities in Williamsville, the suburb of Buffalo to which we moved when I was 3. Then, ambition bubbling to the surface, she returned to college, earned bachelor's and master's degrees in audiology and spent 20 years working as a speech therapist before retiring. She lived to the age of 97, full of energy and opinions until the last few years of her life.

I went to elementary, middle, and high school in Williamsville. Although children of parents with high aspirations are now often sent to private schools, that wasn't the case then, and in fact the Williamsville schools were excellent. When I was in second grade, the teacher suggested to my parents that I be skipped to third grade. After much thought, they consented. In retrospect it was a mistake. I was already small, young for my grade, and physically and socially awkward, all qualities accentuated by skipping. Nonetheless, I generally enjoyed school, had a modest number of friends, participated in a full range of nonathletic extracurricular activities, and spent happy Saturdays and summers helping out at my father's and grandfather's store.

My dad was interested in psychology and psychoanalysis, and among the many books in the house were some collections of case histories jazzed up for general audiences by their therapist-authors—for example, *The Fifty-Minute Hour* (Lindner, 1955). I began reading them in junior high school. These books, discussions of psychology at home, and the novels that I was devouring awoke in me a fascination with mental illness. I even wrote a high school term paper about schizophrenia, in which I described biochemical changes that had been reported to occur in the brain of people with these afflictions (all of which later turned out to be irreproducible). In this way I began transitioning from a literary sort (contributor to the school literary magazine, editor of the school newspaper, and so on) to one with an interest in something that didn't have a name then but that we would now call neurobiology.

Becoming a scientist

High School: Guthrie

Robert (Bob) Guthrie was a microbiologist who grew up poor in the Ozarks, found his way to the University of Minnesota where he earned a PhD and an MD, and then bounced around for several years (NIH, University of Kansas, Staten Island Public Health Hospital, Sloan Kettering) before moving to Williamsville and landing at the University of Buffalo. His research was on the susceptibility of bacteria to purine-based cancer chemotherapeutic agents. When his second child (of six) was born with a severe intellectual disability, he began to think about using his expertise to find ways to diagnose these disabilities.

In early work, he had isolated *E. coli* mutants that required purine for growth (Guthrie, 1949). In a prescient (and awkward) sentence in the introduction of the report, he noted that "the nature of the purine requirement of this organism appeared to be such as to warrant further investigation of its use for assay of natural materials for purine content." I suspect he was thinking of this when he tackled phenylketonuria (PKU), in which mutations of a gene that turns phenylalanine into tyrosine leads to accumulation of high levels of phenylalanine and its metabolites in serum, with devastating effects on brain development. There was already evidence that a low-phenylalanine diet could attenuate symptoms if begun in infancy, but there was no way to screen infants for the defect. Guthrie and Susi (1963) grew *B. subtilis* in the presence of an inhibitor of phenylalanine synthesis. They spotted a few drops of blood from a newborn on filter paper, and placed a small punch on the plate. Bacterial grew only if the blood contained high levels of phenylalanine, with the diameter of the growth ring roughly proportional to the phenylalanine concentration. Guthrie then turned into an advocate, lobbying state legislatures to mandate the test for all newborns. He was eventually successful and now all 50 states require the "Guthrie test."

Another passion of Guthrie's was engaging students in life science research at an early age. To this end, he obtained a National Science Foundation grant and hired a Williamsville High School biology teacher (Mr. Stanford) to run a program. Somehow, Mr. Stanford found and recruited me. The idea was to have us find amino acid auxotrophs (bacterial mutants that would grow only in the presence of specific amino acids) that could be used to generate similar tests for other inborn errors of metabolism. It was a perfect project for young students: technically simple, easily scalable, and requiring no fancy equipment, yet having a valuable goal. I joined the lab, and began working both summers and weekends during the school year.

It was an amazing experience. I was able to do things that would be forbidden today: design, perform, and interpret experiments with minimal oversight; run an autoclave all by myself; and even watch an autopsy on a stillborn infant (for which my medical student mentor was severely chastised). It also introduced me to college students who helped me envision a world beyond Williamsville. And I even did something useful, generating a leucine auxotroph that was later used (sadly without attribution) in a test for Argininosuccinic Aciduria (Murphey et al., 1972). It was called JP3 for Josh and Paula, a student in the program on whom I had an unrequited crush.

College: Greengard

I enrolled at Yale in 1966, the first year in which public school students were admitted in equal numbers to prep school students. Enthused by my experience with Guthrie, my initial idea was to major in biochemistry and find a way to apply biology to brain disorders. However, I quickly realized that one year of math and one year of physics were enough to last me for a lifetime. I then cycled rapidly through several other majors before settling on psychology. I delighted in the stories, especially in abnormal psychology. However, naïve though I was, I felt that there had to be a way to get beyond description to some sort of explanation.

The opportunity arose through a course called Physiological Psychology; it would now be called Introductory Neurobiology. A friend (Bruce Krueger, now a neurobiologist at the University of Maryland) and I were enthralled and approached the professor for advice on how we could do research in this area as seniors. After a few intermediate steps, we ended up in the office of Paul Greengard. Paul had just arrived at Yale at the age of 43, feeling that he had not lived up to his potential and had one last chance to succeed. In fact, he was already quite successful, but that's not how he saw it. At any rate, he had the big idea that mechanisms by which hormones act on target organs—formation of second messengers and stimulation of protein phosphorylation—might also be used by slow or "modulatory" neurotransmitters at synapses. This is obvious in retrospect but was daring at the time

and viewed by many as outlandish. Undeterred, he attacked it with remarkable focus, energy, and creativity. In a series of papers published between 1969 and 1975, he made most of the key discoveries for which he later won the Nobel Prize, showing that slow synaptic transmission is mediated in large part by effects of neurotransmitters on cyclic nucleotides via cyclic nucleotide-dependent phosphorylation of synapse-associated proteins. At the time, I thought this was just what happened in top-tier labs; only later did I realize how unusual it was. (I describe this time in an obituary I wrote; Sanes, 2019.)

Paul invited Bruce and me to join for senior theses. I hatched an ambitious (and unrealistic) plan to unravel the mysteries of the "pink spot," a component of urine visualized on chromatograms, that appeared to distinguish schizophrenics from neurotypicals (Bourdillon et al., 1965). The pink spot had been identified as the dopamine metabolite 3,4-dimethoxyphenyl-ethylamine (DMPEA); dopamine abnormalities had been implicated in the pathogenesis of schizophrenia; and Paul was interested in how dopamine activated adenylate cyclase. So the idea was to find out if those schizophrenics who did and didn't excrete DMPEA formed diagnostically distinct groups (under the supervision of a psychology professor, Ed Zigler) and then find out how DMPEA affected dopamine-sensitive neurons and enzymes. I proposed this plan to a program called "Scholar of the House," which absolved students of course requirements so they could devote the full year to research. To my surprise, I was admitted to the program.

Neither prong worked as planned, but both worked. The collaborator in New York who was going to test patient urine for DMPEA became seriously ill and left her position. However, Zigler arranged for me to visit Bellevue Hospital and go through the records of some 40 schizophrenics to test his hypothesis that developmental level (estimated from indicators such as occupation, level of education, and employment history) was related to symptoms he had characterized as reflecting turning away from the self, turning away from others, or turning against others. Our results supported the hypothesis (Sanes and Zigler, 1970). In Paul's lab, Bruce and I isolated cyclic adenosine monophosphate (cyclic AMP)-dependent kinases from a variety of bovine organs (which we collected from an abattoir) and asked whether they exhibited tissue specific features—which they did (Kuo et al., 1970). In addition, I followed up one difference, that protein kinases from cerebellum were activated by cyclic guanosine monophosphate (cyclic GMP) nearly as well as by cyclic AMP. By fractionation, I obtained the first evidence for a cyclic GMP-dependent protein kinase. More sophisticated work by others was used in the eventual publication, but an abstract reporting the initial finding is the only abstract I list in my CV (Kuo et al., 1969).

Paul was brilliant, funny, lovable, and loving. His lab was still small, and I had much more direct interactions with him than would have been possible a decade later, when the group was an order of magnitude larger.

My experience in his lab gave me not only a taste of exciting science, and a lifelong mentor and friend, but also the credentials to apply to graduate school in neuroscience. Bruce stayed in Paul's lab for his PhD, and I was strongly tempted to do the same, but in the end decided to seek out a new adventure.

Graduate School: John Hildebrand

I applied to the newly formed Departmenr of Neurobiology at Harvard Medical School. They rejected me, but I was accepted into the Department of Biochemistry under the auspices of a separate neuroscience program run by Richard Sidman, who had pioneered the use of mouse mutants (all naturally occurring at that point) to address issues in neural development. I had little to do with the Sidman group, and instead did rotations with Fred Goldberg, who was beginning the studies of protein turnover that subsequently made him the leading figure in this area; and with Larry Okun, an independent fellow working on methods to culture and record from neurons. Larry assigned me the task of testing whether neurons, which are postmitotic, could be provoked to undergo "unscheduled" DNA synthesis following DNA damage. It was exciting to find out that they could (Sanes and Okun, 1972). Larry was one of the smartest and kindest people I ever met, and every minute I spent with him was pure pleasure. In fact, he was so smart that he had been allowed to attend seminars in the Neurobiology Department and sometimes invited me to accompany him. This was a rare privilege, a point I'll come back to shortly.

The rotations were fun, and coursework was enlightening—including a course in molecular biology from Mario Capecchi. (Mario's stay at Harvard was short-lived, in part because he had the audacity to seek mutants in mammalian cells rather than in bacteria or phage as all self-respecting geneticists were supposed to do. After he won a Nobel Prize, Harvard may have regretted letting him go.) Nonetheless I still wanted to be a neurobiologist, and signed up for a rigorous nine-week-long summer course at the Marine Biological Laboratory in Woods Hole, then taught by John Dowling (see Volume 4), Michael Bennett, Rudolfo Llinas, and others. Just as I was leaving, though, the two Neurobiology Department graduate students who had been admitted instead of me left the program and, needing to fill their training grant slots, they admitted me.

The Department of Neurobiology was, at that time, a remarkable place. Founded only a few years earlier, it was chaired by Steve Kuffler and had, as senior faculty, Ed Furshpan, David Hubel (see Volume 1), Ed Kravitz (see Volume 4), David Potter, and Torsten Wiesel. There were also a few junior faculty members, Zach Hall, Jack McMahan, John Nicholls, and Ann Stuart at the time I started, joined by John Hildebrand, Story Landis, and Paul Patterson by the time I finished. The students and postdocs during

that time were a cast of all-stars, including Eric Frank, Charles Gilbert, Jim Hudspeth, Mary Kennedy (see Volume 11), Simon Levay, Louis Reichardt, Carla Shatz, Mike Stryker (see Volume 11), David Van Essen (see Volume 9), and King Wai Yau. The faculty were well aware of their excellence, which had both unpleasant and pleasant consequence. On the downside, the atmosphere was unabashedly elitist, with very few outsiders meriting respect. Work done outside the department was often treated dismissively: only when a result had been replicated in The Department (it was always called The Department) could it be trusted. Seminars, which occurred several times a week at lunch, were not publicized and interlopers were made to feel unwelcome. Indeed, as I mentioned, Larry Okun's admittance, and Gerry Fischbach's later, were rare exceptions. When I joined a lab in The Department, I was asked to renounce membership in Sidman's program and to take the full suite of neurobiology courses. Another consequence of this high self-regard was that all new faculty until around 1990 were chosen from the ranks of departmental students and postdoctoral fellows.

On the positive side, once one was on the inside, the atmosphere was marvelous. Hierarchical distinctions were minimal. The whole Department, including students, postdocs, faculty, and staff had lunch together every day. A set of four semester courses was unparalleled—ones on axon and synaptic physiology taught by Potter and Furshpan, one on neurochemistry by Kravitz and Hall, and one on systems neuroscience by Hubel and Wiesel. Courses were held in the evening, and each week one student gave a lecture on an assigned topic following extensive rehearsal with an instructor (no teaching assistants). As the instructors were all excellent lecturers, we learned presentation skills along with neurobiology. There was hardly any pressure to publish—we were all so sure of our status that there was little need for external validation. Even the junior faculty were spared much of the anxiety that usually accompanies that status. The department had six rather young full professors, and it was clear that no additional tenured appointments would be made for a long time. So the assistant professors could eschew competition (mostly), follow their passion, recruit better students than they would likely get elsewhere, and be assured that the Harvardian glow would earn them a tenured professorship when they had to leave.

My aim was to join Ed Kravitz' lab, collaborate with Paul Greengard, and use Ed's model system, the lobster, to learn more about the role of cyclic nucleotides in synaptic transmission. I would use a leg muscle, which is innervated by both a GABAergic inhibitory neuron (as Kravitz and Potter had demonstrated) and an excitatory glutamatergic neuron. In one set of experiments, I would stimulate one axon or the other, then freeze the muscles and take them back to New Haven to be assayed for cyclic AMP and cyclic GMP. In a parallel approach, I would record from muscle fibers, apply reagents known to mimic or inhibit cyclic AMP or cyclic GMP pathways, and ask how they affected inhibitory or excitatory synaptic potentials.

It was a great idea that failed. I was in over my head and not getting the support an unskilled graduate student needed. In retrospect I suspect that Kravitz was torn between two strong beliefs. One was the egalitarian ethos that compelled him to support a student's desires whatever his own feelings. The other was his skepticism about roles of cyclic nucleotides in neural function. His view—admittedly shared by many neuroscientists at the time—was that the role of cyclic AMP in the brain had already been discovered: it was to modulate metabolism, especially glycolysis, just as it did in other tissues. Cyclic AMP levels were high in brain because it needed a lot of energy. End of story. It all came to a head when I discovered that an exciting result was artifactual, stemming from changes in perfusion flow rate on membrane potential and resistance. When I sheepishly confessed this to Ed he said, oh yes, he had known all about that for years. I was crushed by what I viewed as his lack of concern and realized I needed to get out.

I was torn between finding another advisor and leaving the program. Two conversations with Ed Furshpan helped me recover. In the first, he stood in the stairwell with me for hours after our evening class, and—mostly by listening sympathetically—talked me down off the ledge. In the second, he recommended looking at the arthropod antenna, a rich and pure (neuronally speaking) source of sensory neurons. As luck would have it, John Hildebrand, a postdoctoral fellow with Kravitz, was about to ascend to an assistant professorship and had decided to adopt the holometabolous (undergoes a complete metamorphosis) moth *Manduca sexta* as his model system, so he could address developmental questions. We agreed that development of sensory neurons in the Manduca antenna would be a good starting point for his lab and a plausible path to a PhD for me.

The next few years were happy and productive (which for me are nearly synonymous). Each week I would bicycle to Bio Labs in Cambridge and pick up a small vial of Manduca eggs from a lab that had a breeding colony. I'd bring them back to our lab, lay them on cubes of food that I mixed up, and then transfer them to larger and larger plastic dishes as they passed through their larval (caterpillar) instars, before eventually molting to form pupae. During this metamorphosis, the cellular buds (imaginal discs) that will become appendages evert from inside the larva and become sandwiched between the body proper and the hard protective pupal case. Then, over the next 20 days, a simple epithelial tube develops into a gorgeous antenna, revealed in all its glory when the moth ecloses from the pupa, dries its wings, and flies off.

Sensory organs called sensilla arise by mitosis of epithelial cells; the supporting cells form the cuticular protrusions of various many forms, depending on what modality the neurons are destined to sense (mechanical, olfactory and so on); and the neurons extend axons toward the brain and dendrites into the cuticular sheaths. My job was to follow the process in as much detail and with as many methods as I could muster. I mapped

out the development of the antenna proper, using light and scanning elec-tron microscopy; followed the genesis of sensilla using birth-dating meth-ods (tritiated thymidine autoradiography), and light and transmission electron microscopy; confirmed that the neurons were cholinergic (as had been proposed) and followed the accumulation of the transmitter synthetic and degradative enzymes; and documented the development of responses to odorants using a bulk recording method called the electroantennogram (Sanes and Hildebrand 1975, 1976a, 1976b, 1976c; Schweitzer et al., 1976). And finally, I compared neuronal development in unmanipulated pupae with those in which the brain was excised at the onset of pupation (Sanes et al., 1976). (It turns out that pupae don't need brains to turn into moths.)

Throughout this project, John Hildebrand was an ideal mentor for an ambitious, insecure, and somewhat obnoxious young scientist. He provided me with great independence, gentle guidance, and unwavering support. In return, as his only student or postdoc for the first few years, I was able to help him get his lab off the ground more quickly than would have been possible otherwise. And the detailed description of sensory neuronal devel-opment, although short on major surprises, provided a solid foundation for the beautiful work on olfactory behavior that his lab produced over the following decades. I also ended up getting great help from Jack McMahan, whose lab was down the hall. His expertise (histology) complemented John's (biochemistry), and I sought his advice almost daily as I learned light and electron microscopy—technical help and, as important, how to interpret and not overinterpret what I saw. He had recently begun to work on the NMJ, and our many conversations ignited my interest in that system.

As the project on Manduca wrapped up, I began to look for a lab in which to pursue postdoctoral studies. I contacted several eminences and made a decision. But before starting, I took some time off.

Interludes: Teaching and Policy

Zambia

My freshman advisor, Frank Ballance, spent several years in Uganda and Tanzania in a precursor of the Peace Corps before returning to Yale Law School. After he graduated, he returned to Africa, helping set up the economic ministry in Lusaka, capital of the newly independent Zambia (previously Northern Rhodesia). We stayed in touch and, to my delight, he offered to host my roommate and me during the summer of 1968, after our sophomore year.

It was my first trip abroad, and replete with adventures both en route and there. Frank picked us up in Johannesburg and we visited Soweto (the infamous center of oppression under and resistance to apartheid), then drove South to Lesotho, back North through Botswana and Rhodesia (now Zimbabwe) to Lusaka, passing through game parks along the way.

Once in Lusaka, I started the job that Frank had found for me in the University of Zambia Medical School. It had been founded in 1965 and hadn't yet graduated a class. I worked in the Department of Biochemistry, which was run by a British expatriate named David Mobbs. Actually, he didn't run the department, he *was* the department, so I was able to take on responsibilities that would have been unimaginable under other circumstances—especially since I had not gotten beyond high school biology. I planned, tested, and oversaw the lab sessions for the students. It was a great confidence builder and also my first experience of teaching.

Office of Technology Assessment

I spent most of 1973 cutting sections of Manduca antennae and examining them in the microscope. This coincided with the Senate investigation of the break-in of the Democratic National Committee headquarters by a cadre of Republican operatives, working under the direction of the White House—the so-called Watergate hearings. The hearings were broadcast live and I listened to every word as I cut, stained, looked, and photographed. I was obsessed by the shenanigans and the characters—Sam Ervin, the committee chair ("I'm just a simple country lawyer"); Howard Baker, the ranking Republican ("What did the President know and when did he know it?"); the turncoat Howard Dean ("there was a cancer growing on the presidency"); Rose Mary Woods (the 18-minute gap in the Oval Office tape); the Plumbers; and so much more. I wondered if there might be a way to see some of this stuff up close.

Following his time in Zambia, Frank Balance had returned to the United States and become a staff member for Senator Jacob Javits. He offered to arrange a few interviews, and I ended up taking a position with the Office of Technology Assessment (OTA), a congressional agency that had been set up to provide guidance on scientific and technological issues when called upon by senators, representatives, or committees. It has been established in 1974 with an emphasis on energy policy and was now (late 1975) launching a health policy program. Although I knew nothing whatsoever about health policy, I was an attractive candidate for two reasons: I had a shiny new PhD from *Haahvahd* and I promised to leave in a year.

While there, I worked on two reports. One was on the value of basic research—scarcely a controversial topic, but useful to the Health Subcommittee of the Senate Labor and Public Welfare Committee. The other, more substantive, was requested by the Senate Finance Committee. They wanted to know about the then-new computed axial tomography (CAT) scanner. Because of cost controls in hospitals, physician groups had taken to buying scanners, setting them up in offices near hospitals, and billing insurance for scans. The issue was whether it was sufficiently useful that Medicare should provide reimbursement. As always, if a procedure was

approved, price controls were minimal—what has been called a healthcare system with capitalist supply and socialist demand. But we were asked only about safety and efficacy. We were skeptical initially but as we conducted interviews, read the literature, and attended radiology conferences, we concluded that it had numerous advantages over previous technologies (e.g., pneumoencephalography and arteriography).

The benefits of the year for me were numerous. Most important, I met my wife Susan there. She was also spending a transitional year—in her case between a stint in the Peace Corps and a program in public health. Most immediately, I satisfied my desire to be a voyeur in the federal government, observing the types of people who had fascinated me during the Watergate hearings. A highlight was briefing Senator Edward Kennedy about CAT scanners. I earnestly explained the pros and cons. He listened politely for a few minutes, then interrupted: "Thanks. But what I want to know is who's for it and who's against it." I also learned a bit about science and health-care policy—an interest I have maintained and nurtured, most recently through membership on the National Academies' Committee on Science, Technology, and Law.

OTA also provided me with the opportunity to test my commitment to a life in academic neuroscience research. I had enjoyed the lab enormously but still had a sliver of doubt, and I had entertained thoughts of alternatives such as science journalism. What I found was that I loved the style of policy research but was far less interested in its substance than in learning how the brain worked.

Regarding style, the appeal was irresistible. For example, one day, I called Donald Fredrickson, then director of the NIH, to see if my boss and I could get his views. Had I called six months earlier, I'm sure his assistant's assistant would not have taken my call. Now it was, "I'm so sorry Dr. Sanes, he is completely booked today, but would be happy to meet any time tomorrow that's convenient for you." It's easy to get used to that sort of deference! However, I found myself spending weekends in the library of the George Washington University Medical School, reading journals, taking notes, and thinking about experiments. When September came, I was ready to return to the lab.

The Neuromuscular Junction

Basal Lamina

During the last year of my thesis work, Jack McMahan told me about a remarkable observation he had made. He had been visualizing NMJs in live frog muscles using Nomarski optics and trying to record from them. On occasion, he accidently injured a muscle fiber with his electrode. What he saw was that the cell itself retracted, but the nerve terminal

persisted on what turned out to be the basal lamina that ensheathes each muscle fiber and runs through the synaptic cleft. We called these sheaths "ghosts." Jack's observation revealed what should have been obvious but wasn't: the nerve terminal adheres to the basal lamina, which it contacts directly, rather than the underlying plasma membrane. More intriguing, it made us wonder whether motor axons could be made to reinnervate ghosts following denervation. It had been known since the work of Tello (Cajal's student) that regenerating motor axons selectively reinnervate original synaptic sites, even though those sites occupy less than 1 percent of the muscle fiber surface, a striking example of subcellular specificity in synapse formation. If regenerating axons reinnervated original synaptic sites on ghosts, one would know where to look for the recognition molecules responsible.

I was eager to take this on—it combined my initial interest in synapses with my newer interest in neural development. Before leaving for OTA, I arranged a joint postdoctoral fellowship, in which I would work with Jack to test the idea that regenerating axons would reinnervate initial synaptic sites on basal lamina "ghosts" and, in parallel, work with Zach Hall, another faculty member in the department, to probe the molecular composition of the basal lamina. I had some hesitation about staying in the same department rather than moving to a new environment, but I couldn't imagine anything more interesting than this project. And in fact, Zach Hall had moved to the University of California, San Francisco (UCSF) by the time I got to work, so the two parts of the project ended up being sequential rather than simultaneous, and I did get a chance to experience a different academic environment in a different part of the country.

Over the next year, I worked with another postdoctoral fellow in Jack's lab, Larry Marshall, to test the basal lamina hypothesis. The experiment was conceptually simple. We used a thin flat frog muscle, the cutaneous pectoris, directly beneath the skin of the chest. The muscle fibers ran rostro-caudally and the nerve from lateral to medial along the middle of the muscle, with axons leaving the nerve to deposit a single NMJ on each muscle fiber in a narrow "end-plate band." We would anesthetize a frog, incise the skin to expose the muscle, and cut out "windows" on either side of the nerve, leaving a "bridge" of innervated muscle fibers. If the bridge was wider than 1.5 mm, the muscle fibers would seal off and survive, but if it was narrower, they would degenerate and be phagocytized, leaving a ghost town behind. Resident myoblasts called satellite cells would then divide to generate new myotubes, so in many cases, we x-irradiated the chest (covering the head and legs with strips of lead) to prevent myoblast proliferation and thereby leave the ghosts more or less empty. When we damaged the muscle, we also crushed the nerve lateral to the muscle. The distal axons degenerated but the nerve sheaths persisted, and guided regeneration back to the muscle. The questions then were (1) could we recognize original synaptic sites on

ghosts after the axons and muscle fibers had degenerated, and (2) would regenerated axons reach and preferentially reinnervate these sites?

We recognized the original sites in two ways. One was with a histochemical stain for acetylcholinesterase, which had long been used as a marker for NMJs. (An ancillary benefit was that we demonstrated that acetylcholinesterase was attached to or part of the basal lamina, the first documented association of an enzyme with basal lamina in any tissue; McMahan et al., 1978.) The second was by electron microscopy. In normal muscle, junctional folds invaginate the postsynaptic membrane directly beneath nerve terminals, and struts of basal lamina project into the folds. These struts survived loss of the muscle fiber and its plasma membrane.

By both criteria, reinnervation of original synaptic sites was as precise in the absence of muscle fibers as it was in their presence. We then looked further at the axonal segments that contacted synaptic basal lamina. In normal adult muscle, synaptic vesicles accumulate in nerve terminals, and cluster at active zones that lie directly opposite the mouths of junctional folds. This apposition was reestablished in regenerated axons on ghosts, even including the formation of active zones at sites on the presynaptic membrane that abutted the struts of basal lamina—in other words, where junctional folds had been. Together these results established that at least some of the factors responsible for directing subcellular specificity and presynaptic differentiation, long assumed to be membrane components, were instead stably associated with the basal lamina of the synaptic cleft (Sanes et al., 1976).

The next step was to find those factors—far easier said than done at a time when neither monoclonal antibodies nor recombinant DNA methods had been invented. I moved to Zach Hall's lab, now at UCSF, to get started. With Zach's guidance I tried several approaches. Attempts to isolate synaptic portions of the basal lamina failed completely—no surprise, because it comprised about 0.1 percent of the basal lamina sheath and less than 0.01 percent of muscle extracellular matrix. What did work was to generate antisera from matrices that could be isolated, and then find antibodies within the sera that selectively immunostained synaptic basal lamina. In the end, we found evidence for four distinct synaptic basal lamina antigens—acetylcholinesterase and three others (Sanes and Hall, 1979). These antibodies could then be used to seek bioactive synaptic components.

At this point, early 1979, it was time to look for an independent position. I was very happy in Zach's lab—he remains a good friend—but I was increasingly eager to strike out on my own. Luckily, the work with Jack and Zach had made me fairly marketable, so several opportunities opened up. The two I ended up deciding between were Stanford and Washington University in St. Louis (Wash U). The allure of California was great, as was the possibility of rejoining Jack. Conversely, there were some drawbacks. One was laid out by the chair of the Stanford Neurobiology Department, Eric Shooter.

In a shockingly candid conversation, for which I remain grateful, he said that we would offer me an assistant professorship, but I should realize that if I continued to work on the NMJ, much of the credit for anything I did would accrue to Jack. The other factor was Susan's desire to move Eastward, closer to family (Massachusetts) and farther from what seemed to be a wave of insanity in the Bay Area. In early November 1978, nearly 1,000 cult members who had moved from San Francisco to Jonestown, Guyana, committed mass suicide. Just a few weeks later, Harvey Milk, a city supervisor and gay icon, and George Moscone, the mayor, were assassinated by a disgruntled supervisor.

Luckily, Wash U was attractive in many ways. It was already, and still is, unusually collaborative and collegial. Dale Purves (see Volume 11), a former collaborator of Jack McMahan's at Harvard, took it upon himself to recruit me to the Department of Physiology, which he had joined a few years earlier. He instilled confidence as did the chair, Cuy Hunt (see Volume 5), who had already built successful departments at Yale and Utah and had the perfect light touch. There was also a long and glorious tradition of neuroscience—among some 20 Wash U Nobel laureates were Joseph Erlanger and Herbert Gasser, who characterized sensory nerves, and Rita Levi-Montalcini and Stanley Cohen, who discovered nerve growth factor (NGF). At the time I was recruited, chairs of three medical school departments were neuroscientists—Cuy in physiology, Luis Glaser in biochemistry, and Max Cowan (see Volume 4) (who left before I arrived) in anatomy, as well as Viktor Hamburger (see Volume 1), who had stepped down as chair of biology but remained a powerful presence. Indeed, although I never grew to love St. Louis, I never ceased to love Wash U.

The first order of business at Wash U was to learn more about the basal lamina. My first postdoctoral fellow, Arlene Chiu, generated monoclonal antibodies that defined three groups of basal lamina components: those concentrated at synaptic sites, those excluded from synaptic sites, and those present throughout the basal lamina (Sanes, 1982; Sanes and Chiu, 1983). Arlene also obtained initial evidence that one synaptic component, recognized by an antiserum and a few monoclonal antibodies (C1 and C4; C for Chiu), was a protein of about 190 kD that was stably associated with basal lamina but could be solubilized by denaturing and reducing agents.

Dale Hunter, a postdoctoral fellow who joined the lab in 1985, took the next steps. He noted that C1 and C4 stained not only synaptic basal lamina but also glomerular basal lamina in kidney, a vastly richer source. He used glomeruli to generate another set of monoclonal antibodies (D5 and D7 for Dale) that he used to purify the protein and show that neurons were adherent to it. Most important, we collaborated with John Merlie—about whom more later—to isolate cDNAs encoding the antigen, using expression cloning in bacteria. Following several rounds of screening (in each case using a recovered partial cDNA to identify clones that extended further), we

ended up with the sequence of a protein that was clearly related to laminin (Hunter et al., 1989a). This was interesting for a few reasons. First, laminin was a known component of basal laminae. It was a heterotrimer of three subunits, initially called A, B1, and B2 and subsequently renamed α1, β1 and γ1. Our protein, which we called synaptic laminin or s-laminin (pronounced slaminin) expanded the laminin gene family; it was later renamed (to my distress) β2. We and others later showed that there are some 5α, 3β, and 3γ chains that can assemble in multiple αβγ trimers. Second, and more relevant here, the initial α1/β1/γ1 laminin had already been shown to support neuronal adhesion and promote neurite outgrowth. It made sense that a laminin variant could act in a related but distinct way. Indeed, Dale and Brenda Porter, an MD-PhD student, showed that s-laminin bore a small adhesive domain that was selective for motor neurons and that could act to inhibit the growth that classical laminin promoted—in other words, while classical laminin made axons grow, s-laminin made them stop growing and start differentiating (Hunter et al., 1989b; Porter et al., 1995).

The discovery that there were multiple laminins with a special form in synaptic basal lamina raised the possibility that the same was true for other basal lamina components—collagens IV, heparan sulfate proteoglycans, and entactins/nidogens. We tested this idea over the next several years and found that it was correct. In fact, basal laminae are much more variable than had previously been thought, with differences among cells in a tissue, transitions in isoform composition during development, and compensatory changes in mutant mice lacking specific isoforms (e.g., Sanes et al., 1990; Miner and Sanes, 1994, 1996; Miner et al., 1995, 1997; Patton et al., 1997; Fox et al., 2008). Thus, although our focus remained on the NMJ, the analysis, and the conclusions we drew from it, extended to many tissues and organs. In particular, several of the mutant mice we generated (including *lamb2* mutants) had lethal glomerular (kidney) defects (Noakes et al., 1995b; Miner and Sanes, 1996) leading Jeff Miner, who had led much of this work, to abandon neuroscience in favor of nephrology; he now leads the nephrology research division at Wash U.

Collaboration with John Merlie

Our entrée to the synaptic cleft was through studies of reinnervation following injury. The second order of business at Wash U (once biochemical analysis was underway) was to look at development: how the basal lamina formed, how its components were regulated by neuromuscular interactions, and what roles (if any) they played in synapse formation and maturation. We documented the series of steps by which the basal lamina formed (Chiu and Sanes, 1984) and collaborated with John Lawrence (a new assistant professor in pharmacology) to show that activity and nerve-derived factors regulate basal lamina deposition in cultured muscle (Sanes and Lawrence,

1983; Sanes et al., 1984). Functional studies, however, required a different approach. A life-changing collaboration with John Merlie made that possible.

By the time John was recruited to Wash U in 1982, he was already a major contributor to studies of the nicotinic acetylcholine receptor (AChR), the neurotransmitter receptor concentrated in the postsynaptic membrane of the NMJ. He worked with pioneers in the area, Jean-Pierre Changeux at Institut Pasteur in Paris and Steve Heinemann and Jon Lindstrom at the Salk Institute in La Jolla, before moving to a faculty position at the University of Pittsburgh, where he began applying recombinant DNA methods to the problem. He was soon tenured there, but Phil Needleman, the chair of pharmacology, persuaded him to move without tenure, and trust that it would come soon—which it did.

John and I had our first long talk in 1983 on a plane taking us to Cold Spring Harbor for their annual symposium, at which we were both speaking. It was on molecular neurobiology that year and served as a sort of coming out party for what was then an emerging field. We quickly realized that our interests were similar (synapse formation at the NMJ) but slightly different (presynaptic vs. postsynaptic) and that our distinct skill sets would allow us to do experiments together that neither of us could do alone. Our first foray was to show that denervation supersensitivity, the appearance of AChRs in extrasynaptic membrane following denervation or paralysis, was preceded by, and presumably due to, an increase in AChR subunit mRNA (Merlie et al., 1984). What I remember most vividly about this study was that after the experiments were complete, we had to hold the manuscript for a few months until we obtained 80 base pairs of sequence to verify that the cloned cDNA probe indeed corresponded to the AChRα subunit. What a contrast to experiments I'll describe below in which more than 10 billion base pairs of sequence are delivered to our hard discs overnight—a rate increase of more than 10^9-fold.

In our next study, we asked whether there might be transcriptional contributions to the concentration of AChRs in the postsynaptic membrane of normal adult muscle. I used microscissors to dissect end plate-rich and -free portions of diaphragm—not unlike what I had done to make bridges of basal lamina ghosts eight years earlier, except in this case from dozens of mice per experiment. John then generated RNA from each sample and probed it on Northern blots with cDNAs encoding two AChR subunits. In both cases, levels of mRNA were several-fold higher in the synaptic sample than in the extrasynaptic sample (Merlie and Sanes, 1985). Because the synapse comprised only a small fraction of the nominally synaptic sample, we considered that perhaps the true difference was much greater. Diaphragm muscle fibers contain about 500 nuclei of which about five lie directly beneath the postsynaptic membrane. Thus, only 3 percent of the nuclei in the synaptic third were actually synaptic, so even if they transcribed AChR genes at 100x

the rate of extrasynaptic nuclei, the difference between sample would only be a few-fold. We therefore speculated that synaptic nuclei were transcriptionally specialized, implying that the nerve acted not only to determine where AChRs accumulated but also where they were synthesized. Later we generated transgenic mice in which regulatory elements of AChR subunit genes drove expression of a nuclear-localized reporter and showed that indeed transcription was largely confined to synaptic nuclei (Sanes et al., 1991; Gundersen et al., 1993). We also used in situ hybridization (which had failed in 1985) to show that several other components of the postsynaptic membrane are synthesized locally (Moscoso et al., 1995). Later still, Masashi Kishi, a postdoctoral fellow, used microarrays to identify mRNAs concentrated in synapse-rich samples, identifying novel components of the postsynaptic membrane (Kishi et al., 2005a). Thus, muscles, like some neurons, harness local protein synthesis to help sculpt the postsynaptic membrane, but by different mechanisms—localized transcription in the multinucleated muscle fiber and selective RNA transport to dendrites in neurons.

As this work was proceeding, John had become proficient in the generation of transgenic mice. In this method, developed by Richard Palmiter and Ralph Brinster in the early 1980s, DNA is injected into the pronucleus of a fertilized mouse egg, which is then implanted in the oviduct of a pseudopregnant mother—much as is now done with people. The transgene integrates at random into a chromosome and essentially becomes part of the genome. John initially used the method to explore regulatory elements of AChR genes in bulk assays (Merlie and Kornhauser, 1989) and soon, as noted earlier, with reporters that could be visualized (Sanes et al., 1991). Around 1990, he decided to take the brave next step of importing knock-out technology, in which endogenous genes could be mutated—the method that Mario Capecchi coinvented after leaving Harvard, and for which he shared a Nobel Prize. The first reports on generation of such mice appeared in 1989, and it was immediately clear that the method would be transformative: one could, for the first time, choose which gene to mutate and, soon, what mutation to make (knock-in mice). This was not even possible in Drosophila! It was, however, very difficult. Among the many steps, perhaps the most challenging were learning to cultivate embryonic stem cells without having them differentiate or get contaminated, and generating vectors at a time when neither PCR nor genome sequence was available.

Two postdoctoral fellows—Peter Noakes in my lab and Medha Gautam in John's—rose to the challenge. We decided to mutate three genes— s-laminin (lamb2); rapsyn, an AChR-associated scaffolding protein that John had cloned and studied; and agrin, a nerve-derived basal lamina-associated organizer of postsynaptic differentiation that Jack McMahan and colleagues had discovered. They initially used the "bridge" assay that had taught us about subcellular specificity of reinnervation, but in this case, they allowed myotubes to regenerate in persistently denervated basal lamina "ghosts."

Steve Burden in Jack's lab found that AChRs clustered in the new myotubes directly beneath original synaptic sites, showing that basal lamina could direct both pre- and postsynaptic differentiation (Burden et al., 1979). Based on this finding, they purified the aggregating factor, and eventually both McMahon's group and Scheller's, also at Stanford, cloned it (McMahan, 1990). Along the way, we established a collaboration with Yancopoulos and colleagues at Regeneron, who had isolated muscle-specific receptor tyrosine kinase (MuSK), which turned out to be a critical subunit of the agrin receptor. We believed that together these might comprise the core components of a neuromuscular synaptogenesis program.

Progress was slow. I threw most of my NIH funding behind the effort, as did John. Eventually we applied for a program project grant from NIH to allow us to proceed. We had precious few "preliminary results," but I wrote that if our application was turned down, this new technology would be available only to investigators in "Highly Huge Medical Institutes." Whether for this reason or another, we were funded.

Perhaps most disheartening was agrin. Vectors had to be made from the genomic clones that could be recovered, and we used one in which we could delete a set of exons in the 5' (N-terminal) portion. The mutant mice were horribly normal. It turned out that the deletion was in a region encoding multiple copies of an apparently dispensable domain (called a follistatin repeat), and the mice were able to make a protein that lacked these exons but was nonetheless functional. We therefore went back and deleted a few more exons—and got the same result. Finally, in what would surely be the last try, we succeeded in inactivating the gene. In quick succession, we obtained laminin β2, rapsyn, and agrin mutants, and the Yancopoulos group obtained MuSK mutants, which we subsequently analyzed together. All were lethal— agrin and MuSK at birth, rapsyn shortly thereafter and laminin β2 a few weeks later—demonstrating, in combination with other observations, that they were essential for neuromuscular synaptogenesis (Noakes et al., 1995a; Gautam et al., 1995, 1996; DeChiara et al., 1996; Apel et al., 1997; Zhou et al., 1999). Our bet had paid off.

With this success we began thinking about next steps—more genes, more analyses, and forays into the central nervous system. Then disaster struck. John and I usually got together on Saturday mornings to discuss progress and make plans. On Saturday May 27, 1995, we were particularly excited, because we were going to dissect a litter of agrin knockout mice and image NMJs. As I prepared to drive into the lab, the phone rang. John had suffered a heart attack and died almost immediately. In a moment, I lost a dear friend and my closest collaborator.

It was a horrible time. In the few months after John's death, I lost my father to a glioblastoma, one of my best friends from college to AIDS, and my grandmother to the ravages of old age. My wife became extremely ill from a large abdominal infection and its sequelae. And overnight the size

of my group doubled, as I stepped in to mentor John's students, fellows, and research assistants, and to supervise the transgenic facility that he had recently established. It also marked the end of my having time to do any experiments with my own hands.

Formation and Maturation of the Neuromuscular Junction

Time—and the unsurprising depression that followed—passed, and both scientific and family life recommenced. Particularly important was the strong support from Wash U, which allowed John's lab to remain open until we could find new homes for his group members. A few joined my group, a few completed their theses and moved on, a few got other positions, and so on. One senior research assistant, Mia Wallace, took over the transgenic facility, which survived my year as the weekend staff member; she eventually built an institute-wide facility that she supervises to this day. Eventually, we returned to the genetic analysis of neuromuscular synaptogenesis that John had begun. Over the years, we generated (or, in some cases, obtained) mutants in more than 40 genes and analyzed their roles in the formation, maturation, and maintenance of this synapse. I believe this remains the most complete genetic analysis of development for any synapse, and it laid the foundation for studies of central synapses by numerous other groups.

I'll mention three sets of studies from this long series here. Most related to the questions I initially set out to answer was a detailed analysis of muscle-derived factors that organize presynaptic differentiation. We analyzed roles of several laminin subunits, showing distinct roles for the β2, α4, and α5 chains. In brief, β2 appears to promote maturation of nerve terminals (Noakes et al., 1995a; Fox et al., 2007), in part by binding to calcium channels in the presynaptic membrane (Nishimune et al., 2004); it also actively excludes glial (Schwann) cells from the synaptic cleft (Patton et al., 1998). α4 is responsible for the precise apposition of active zones with junctional folds (Patton et al., 2001), and α5 has autocrine functions in postsynaptic maturation (Nishimune et al., 2008).

We also sought roles for neural cell adhesion molecule (NCAM), Tenascin-c, fibroblast growth factor 5 (FGF5), and ColQ, the collagenous subunit of acetylcholinesterase. However, despite discovering many interesting features of their localization and regulation, we obtained no evidence for major roles in synaptogenesis (Covault and Sanes, 1985; Covault et al., 1986; Sanes et al., 1986b; Gatchalian et al., 1989; Moscoso et al., 1998; Feng et al., 1999). This was disappointing but illustrates a great advantage of the genetic approach: one can test candidates nominated based on, for example, expression patterns or bioactivity in cell culture and then triage poor candidates to focus on the critical ones. Eventually, Hisashi Umemori, later joined by Mike Fox, took a more direct, unbiased approach. Hisashi devised

a bioassay based on clustering of synaptic vesicles within cultured primary neurons and then used it to monitor purification of active material from brain and Torpedo electric organ. This approach yielded three sets of molecules: a group of FGFs (7, 10, and 22), a signal regulatory protein (SIRPα), and cleaved terminal domains of several collagen subunits (Umemori et al., 2004; Fox et al., 2007; Umemori and Sanes, 2008). Hisashi and Mike have continued to pursue roles of FGFs, SIRP, and collagens in their own labs (e.g., Terauchi et al., 2010; Toth et al., 2013; Su et al., 2012).

A second set of studies following up the analysis of postsynaptic differentiation was initiated with Merlie. We used targeted mutagenesis to selectively delete several alternatively spliced exons in agrin whose existence had been noted but whose function remained unclear. In this way, we found that one short exon near the C-terminus was essential for its AChR-aggregating activity (consistent with previous work in tissue culture), whereas several other segments were dispensable (Burgess et al., 1999, 2000). Surprisingly, a synapse-specific carbohydrate that we had identified much earlier (Sanes and Cheney, 1982) also affected AChR clustering (Martin et al., 1995). Of several components of the postsynaptic membrane and cytoskeleton that we studied, the most interesting were members of the dystrophin-associated glycoprotein complex (DGC) that Kevin Campbell had isolated in his studies of Duchenne muscular dystrophy, caused by loss of dystrophin. We followed up on hints that—just as had been the case for laminins and collagens—the DGC differed between synaptic and extrasynaptic membrane, with different members of gene families and/or different alternatively spliced isoforms in the two regions. An autosomal homologue of dystrophin, called utrophin, is concentrated beneath the postsynaptic membrane. Loss of either utrophin or dystrophin had only a modest effect in mice—surprisingly, given the lethality of dystrophin loss in humans. What we found was that in mice, utrophin compensates for loss of dystrophin, with double mutants faithfully recapitulating virtually all aspects of the Duchenne phenotype (Grady et al., 1997). These double mutants have since been used in preclinical tests of potential therapies in mice. As for other synaptic DGC components, they appear to be dispensable for initial formation of the postsynaptic membrane but are crucial for its postnatal maturation (e.g., Grady et al., 2000, 2003).

Third, we used genetic methods to explore roles of electrical activity in synapse formation and maturation. Our reagent was a mutant lacking choline acetyltransferase (ChAT), the enzyme that synthesized the neurotransmitter acetylcholine; in the absence of ChAT, neuromuscular transmission was abolished, and the mice died at birth (Misgeld et al., 2002). With this assurance, we performed two experiments. In one, we studied the role of activity in synapse elimination. In early postnatal life, individual muscle fibers are innervated by multiple axons, and then all but one withdraw, resulting in the adult pattern of single innervation. It was abundantly clear that elimination was driven by activity, but it was less clear whether it

was the total or relative activity of the inputs that mattered. By inactivating some or most inputs (using a conditional allele), we showed that the more active axon won the competition; when both (or all) axons were inactive, multiple inputs persisted (Buffelli et al., 2003).

The second experiment helped resolve a paradox that had arisen with respect to how pre- and postsynaptic specialization come to be apposed to each other. The dogma was that axons grow into the center of a muscle and secrete agrin to generate postsynaptic specializations in the central endplate band. However, we and others found that the endplate band formed when agrin—or even innervation—was missing (Yang et al., 2001; Lin et al., 2001), and in some cases, axons grew toward preexisting postsynaptic specialization (Flanagan-Steet et al., 2005). By studying double mutants lacking both agrin and choline acetyltransferase, we found that synapses form in the absence of agrin provided that acetylcholine is also absent. Our conclusion from these and other experiments was that activity destabilized postsynaptic specializations unless agrin is present; that is, a major role of agrin is to give the "correct" postsynaptic structure immunity from the dispersing effect of activity (Misgeld et al., 2005). I found this conclusion especially pleasing because it was completely consistent with essentially all previous results of many groups—but provided a strikingly new interpretation (Kummer et al., 2006).

Imaging

With the initial aim of visualizing NMJs, Guoping Feng generated a large variety of transgenic lines that expressed various fluorescent proteins (XFPs) in motoneurons. This is fairly routine today, but at that time (around 1997), there were many fluorescent proteins and many regulatory elements that needed to be tested in many combinations. The result was a set of XFP lines expressing green, yellow, blue, or red fluorescent proteins in a variety of patterns—some, for example, in all motoneurons and others in just one or two per muscle (Feng et al., 2000b). Most also lit up other neuronal populations, and have been used in literally hundreds of studies by dozens of laboratories; Guoping's paper has now been cited more than 2,000 times. Later, Jean Livet, a remarkably creative postdoc, took this approach a step further, inventing the "Brainbow" method that enables marking neurons in a range of dozens to hundreds of colors (Livet et al., 2007; Cai et al., 2013). Although frankly less useful than the XFP lines, they generated far more beautiful micrographs, which have now appeared in museums throughout the world.

Jeff Lichtman, an expert in imaging methods, helped in the initial characterization of the XFP lines and cosupervised development of the Brainbow method. Jeff was a postdoctoral fellow with Dale Purves, left Wash U for a short postdoctoral fellowship, and then returned to join the faculty shortly after I arrived. We quickly became friends and eventually collaborated on a

variety of studies using the XFP lines to investigate neuromuscular development, regeneration, disease, and aging (e.g., Nguyen et al., 2002; Schaefer et al., 2005; Valdez et al., 2010). Jeff had (has) a deep suspicion of studies with molecular goals or that use manipulations to investigate mechanisms, so our points of view couldn't be more different. Although he failed to change my mind in hundreds of conversations over some three decades, his influence on my thinking was profound. We also combined our contrasting views productively to comment on issues that spanned these areas (Sanes and Lichtman, 1999; Lichtman and Sanes, 2008).

Interlude: Lineage

I had long wanted to spend time in France, and even began arranging a postdoctoral fellowship with Jean-Pierre Changeux (see Volume 4) before being seduced by the basal lamina. An opportunity arose in 1985, thanks to a junior faculty grant called an Established Investigatorship of the American Heart Association, which encouraged recipients to spent protracted time in another lab. Medical schools in general don't have clear sabbatical policies, but I argued successfully that as long as they weren't paying my salary they shouldn't tell me where to sit. Changeux was still the obvious choice, but I wanted something less related to my current work. I asked John Merlie, who had loved his years in Paris, for advice, and he suggested I contact Francois Jacob. I did so, and arranged an interview.

Jacob was courtly and kind when I visited, and said he'd be willing to host me. I found an apartment—a former *chamber de bonne* (maid's quarters) atop an apartment building a few blocks from the Pasteur. It was wonderful—recently renovated with a clear view of the Eiffel Tower from one window, on a quiet side street, and just one block from a Saturday farmer's market. In August, Susan and I moved in.

By 1985, although he continued to preside over a unit that filled a floor of "Batiment Monod," Jacob was rather disengaged from science. At our first meeting, he suggested I visit the junior faculty arrayed across the floor, each with a few rooms, a few students, and a few technicians, choose one and let his secretary know my decision. Over the next year, he took me to lunch at a nearby café every few months, where we had omelets and frites, and made awkward conversation, but we seldom talked about science. I later found out that he was writing his wonderful autobiography, *La Statue Interieure* (*The Statue Within*) at the time; much of it described his childhood, life in the French Resistance, and the war wound that dashed his hope of becoming a surgeon—so it's easy to imagine that he was lost in that world.

After some wandering, I joined the group of Jean-Francois Nicolas and began working with John Rubenstein. Jean-Francois had worked on teratoma cells (an early version of embryonic stem cells) as a PhD student with Jacob and then spent a few years as a postdoctoral fellow at Stanford before

returning to Pasteur to apply molecular methods to early embryogenesis. John had earned an MD and PhD at Stanford and then joined Jean-Francois as a postdoctoral fellow; he is now an eminent neuroscientist at UCSF. John and I quickly became good friends and have remained close ever since.

At the time, Jean-Francois and John were trying to generate transgenic mice by infection of early embryos with a recombinant retrovirus containing the bacterial beta-galactosidase (lacZ) gene—which Jacob and Monod had used long before in their discovery of the operon. I spent a few weeks trying to demonstrate lacZ activity in transgenic embryos, but it soon became clear that the method was doomed to failure—because, we now know, the viral regulatory sequences are silenced by methylation early in embryogenesis. Seeking to find a better use for the virus, Jean-Francois came up with the idea of using it to trace cell lineage at later embryonic stages. We would infect single cells in postimplantation embryos, the retroviral genome would be integrated into the host genome, and we would be able to track progeny over many generations, based on their expression of lacZ. John was heading back to California, but I thought it was a wonderful idea and took it on.

The first step was to devise a histochemical stain for lacZ. A substrate that generated a blue product, X-gal, had long been used to stain bacterial colonies, but it took weeks of fiddling to find ways to immobilize the product as soon as it was generated, so that it would provide cellular resolution and survive tissue processing. Next, I used cultured cells to show that infection had no adverse effects on proliferation, that the regulatory elements in the virus were active in a wide variety of cell types, and, most important, that expression persisted over many cell divisions, indelibly marking the progeny of the infected cell. Once that all worked, I injected minute amounts of virus into early mouse embryos, waited several days, then looked for groups of lacZ-positive cells—putative clones. I was able to recover many cell clusters in yolk sac and skin, accumulate evidence that the clusters were indeed clones and, by comparing clones at various intervals following infection, reconstruct cell lineages (Sanes et al., 1986). As far as I know, this provided the first way to trace postimplantation cell lineages in a mammal, enabling a rigorous comparison of actual cell fate with the range of potentials a cell could exhibit in culture or after transplantation. Clearly, the combination of information about fate and potential was far more revealing than either alone.

In these first experiments, I had little success infecting cells in the brain. Once I got home, I was eager to try again. I decided to use the chick embryo, because it was much easier to expose and target the neural tube when the creature was surrounded by an egg rather than a mother. In fact, Marla Luskin, an extremely talented neuroanatomist, soon found ways to study the mouse cortex (Luskin et al., 1988), but it was much more difficult, so we largely stuck with chick.

The viral vectors I had used in France did not infect chick cells, but we were able to adapt (repackage) them. We also collaborated with John

Majors, who had studied avian tumor viruses with Mike Bishop and Harold Varmus at UCSF before joining the Wash U faculty. He was extremely smart and absolutely adored cloning, and soon generated several new vectors. We initially used the optic tectum, because it is large, prominent, and has a very orderly arrangement of cells and laminae. Grace Gray, a graduate student and two postdoctoral fellows, Joel Glover and Deni Galileo, showed that single tectal progenitors could give rise to nearly all major tectal neuronal classes as well as glia (Gray et al., 1988; Galileo at al., 1990). Connie Cepko, who had devised essentially the same method at the same time, reached a similar conclusion in rodent retina (Turner et al., 1990). Tracing the distribution of cells in the clones over time also helped us understand the migratory paths that neurons take on their way from their birthplace in the ventricular zone to their final laminar destinations (Gray and Sanes, 1991). In the course of this work, we obtained some insight into the then-elusive radial glial cells, making the case that they are in fact the tectal stem cells (Gray and Sanes, 1992)—which was later shown to be the case in mammalian cortex as well (Fishell and Kriegstein, 2003). We also applied the method to spinal cord (Leber et al., 1990) and sensory ganglia (Frank and Sanes, 1991).

By 1990, I began to phase out this work. One problem was that it was fundamentally unrelated to the project on neuromuscular synaptogenesis, and I found it unpleasant to have two subgroups in the lab with little interest in each other's work and little possibility of working together. In addition, it was becoming clear that further progress would require moving beyond description to elucidate the transcriptional programs that underlie fate choices—a path that Connie Cepko (then a competitor, now a friend and colleague) has followed with great success. That was not a prospect I found too attractive, especially when the collaborative work with Merlie was so exciting and fun. I remember telling a colleague about our work on lineage around that time. He said, "that's interesting, but I think of you more as a synapse guy." He was right.

Synaptic Specificity

My core interest has been in how neurons make the right connection—synaptic specificity. Studies on the basal lamina began with a focus on subcellular specificity. Once I got to Wash U, along with pursuing that theme, I was eager to move on to the cellular specificity that accounts for the stereotypy of neural circuits.

Dale Purves provided an entrée. He had been using physiological methods to find the cellular basis for a result that J. N. Langley (in my opinion the second greatest neurobiologist of all time, after Cajal) had obtained long before (Langley, 1895). Langley studied the innervation of peripheral targets of sympathetic ganglia that were supplied by preganglionic neurons from

different spinal levels. He found that neurons innervated from rostral or caudal spinal segments preferentially innervated rostral or caudal targets, respectively. He then severed the preganglionic nerve and waited for regeneration. What he found was that this segmental preference was reestablished, leading him to propose a molecular explanation that predated Roger Sperry's chemoaffinity hypothesis by more than 50 years. Purves confirmed Langley's result at a single cell level and then, with Wes Thomson and Joe Yip, performed an experiment that I view as his best ever (although I know he will disagree violently). They transplanted ganglia from various segmental levels to the bed of the superior ganglion, and showed that ganglia from rostral or caudal levels were preferentially innervated by preganglionic axons running through rostral or caudal ventral roots, respectively (Purves et al., 1981).

Langley had also shown that autonomic preganglionic axons could innervate skeletal muscle fibers, so we wondered if such connections would also exhibit positional selectivity. Don Wigston, who had been a postdoctoral fellow with both Dale and Viktor Hamburger, joined the lab to test this idea. He transplanted intercostal muscles from different segmental levels to a common site and sutured in the preganglionic trunk. He then dissected the muscle in contiguity with ventral roots (an arduous procedure that he taught me so we could both do experiments) and recorded from muscle fibers to determine the segmental origin of their inputs. As was the case for ganglia, rostral and caudal muscles were preferentially innervated by axons from rostal and caudal levels, respectively (Wigston and Sanes, 1982, 1985), suggesting the existence of a body-wide program of positional information. Mike Laskowski, a faculty member at St. Louis University who came for a sabbatical, later showed that the mapping function was a bona fide part of neuromuscular development, not some peculiarity of the cross-innervation paradigm: in muscles innervated from multiple spinal segments, the rostrocaudal axis of the motor pool was systematically mapped onto the rostrocaudal axis of the muscle, and the map was reestablished following denervation and reinnervation (Laskowski and Sanes, 1987, 1988).

How to go forward? A serendipitous observation provided a way. Maria Donoghue, a graduate student in Nadia Rosenthal's laboratory at Boston University, had characterized regulatory elements from a myosin gene that Merlie used to generate transgenic mice (Rosenthal et al., 1989). Nadia asked if Maria could visit our labs to characterize patterns of transgene expression. Maria and I began assaying many different muscles and found, to our surprise, that levels of reporter varied among muscles in several independently derived transgenic lines, forming a 100-fold gradient in which a muscle's position in the rostrocaudal axis was correlated with its level of reporter expression (Donoghue et al., 1991). Although we never fully understood the basis of this peculiar pattern, it provided a useful marker: by generating muscle cultures and even immortalized cell lines from the

transgenic mice, we could show that the muscles bore a cell-autonomous, heritable memory of their rostro-caudal position that was maintained by transgene methylation (Donoghue et al., 1992a, 1992b).

The implication was that this positional memory might drive expression of a recognition molecule that accounted for positionally selective innervation. Maria therefore used a subtractive hybridization approach to look for genes differentially expressed between rostral and caudal muscles and muscle cell lines, and came up with a great candidate. Disappointingly, while she was cloning her way through a ridiculously long untranslated region to reach the protein-coding sequence, the same gene, ephrinA5, was discovered and implicated in retinotectal mapping—also along the rostrocaudal axis— by the Bonhoeffer and Flanagan groups (Cheng et al., 1995; Drescher et al., 1995). On the bright side, the parallel spurred our efforts to implicate ephrins in the positional selective innervation of muscle. In favor of that idea, we found that recombinant ephrinA5 inhibited outgrowth from cultured motoneurons in a position-dependent manner and that manipulation of ephrin A5 in mice (by knockout or transgenic overexpression) degraded the topographic motor maps (Donoghue et al., 1996; Feng et al., 2000a).

This series of studies was deeply gratifying, but I was increasingly concerned that the effects we saw were quantitative rather than qualitative—highly significant statistically but scarcely overwhelming. The reason may be that once a motor axon is guided to the appropriate target muscle it doesn't care all that much which muscle fiber it innervates. Specificity matters more in the brain, where highly stereotyped patterns of connectivity underlie neural circuitry. So, we had to study the brain itself.

Retina

Studies in Chick Embryos

The first steps in this project were taken by Masa Yamagata, a postdoctoral fellow with perhaps the greatest range of skills and most astonishing work ethic of anyone who has ever been in my lab. After a discouraging attempt to find more "neuromuscular specificity" genes, we decided to tackle the retinotectal projection in the chick embryo. There were two reasons. First, it was familiar territory from the studies on neuronal lineage and migration described above. Second, it had long been a favorite preparation for studies of axon guidance, culminating in the discoveries of ephrins and eph kinases, so a lot was known about the projection. Our idea was to look at laminar specificity—the phenomenon whereby once the axons of retinal ganglion cells (RGCs, the sole output neurons of the retina) arrive at their proper position along the dorsoventral and rostrocaudal axes, they dive into the neuropil to form synapses in specific tectal laminae. Masa began with a comprehensive inventory of the expression of "known" candidate adhesion

molecules (Yamagata et al., 1995) and then adapted a "cryoculture" system that we had devised in studies on the NMJ (Covault et al., 1987). In this method, neurons are cultured on cryostat sections of a target tissue, and the trajectories of their axons assessed. Masa found that outgrowth, branching, and arborization patterns of retinal axons on tectal slices were lamina-selective, indicating the existence of localized cues that guide retinal axons (Yamagata and Sanes, 1995). We made modest progress over the next years in identifying a few of these cues (Inoue and Sanes, 1997).

Masa returned to Japan in 1995, and established a group at an institute in Okazaki. However, despite substantial success, he was unhappy in that position, so I suggested he return to Wash U for a respite while he figured out what to do next. He did return, took up where he had left off, and ended up staying through 2020. He began by looking for recognition molecules that might explain how different types of RGCs terminate in different tectal laminae, where they synapse on different target cells. To this end we contacted Catherine Dulac, who had invented a method for generating cDNA libraries from single neurons and differentially screening them to isolate cell type–specific genes—in her case, genes encoding vomeronasal receptors (Dulac and Axel, 1995). Catherine agreed to help, provided we promised to follow her protocol exactly—if we deviated and ran into problems, she would cut us off. I was in complete agreement with this restriction, having learned from Zach Hall that when trying a new method, one must begin by slavishly following the established protocol before trying to get clever and improve matters. I have preached the gospel of "slavish following" many times over the years—with mixed success.

Masa generated single cell libraries from RGCs that differed in size and surface properties and then used differential screening to find genes selectively expressed by one or another of the cells. He ended up with two closely related genes that encoded large transmembrane molecules with features that placed them in the "immunoglobulin superfamily," a group known to include numerous recognition and adhesion molecules. We called them Sidekick-1 and -2 based on very weak homology to a Drosophila gene. With Josh Weiner, Masa showed that they were expressed by nonoverlapping subsets of RGCs (perhaps 20 percent each) as well as by subsets of retinal interneurons, that the Sidekick proteins were concentrated at synaptic sites, and that they both mediated homophilic adhesion—that is, beads coated with recombinant Sidekick-1 adhered to other Sidekick-1 bearing beads but not to Sidekick-2 bearing beads and vice versa (Yamagata et al., 2002). These results suggested that the Sidekicks could indeed promote differential connectivity of RGC subsets with synaptic partners.

When Masa and Josh Weiner began looking at the morphology of the RGCs that expressed each Sidekick, we had a surprise. In the retina, interneurons called bipolar and amacrine cells project to a synaptic layer called

the inner plexiform layer (IPL), where they synapse on dendrites of RGCs. The IPL can be divided into numerous sublaminae, with each interneuronal and RGC type arborizing on only one or a few of the sublaminae. This laminar specificity is akin to the laminar specificity we had been studying in tectum. What Masa and Josh found was that processes of both interneurons and RGCs that expressed Sidekick-1 projected primarily to the same sublamina, and Sidekick-2 positive cells projected to a different sublamina. We realized at once that we should be looking at specificity in the retina itself rather than in the tectum, and soon found that overexpression or depletion of Sidekicks led to redistribution of arbors in predictable ways (Yamagata et al., 2002; Yamagata and Sanes, 2008). Masa also went on to show similar patterns of expression and function for several related molecules, called Dscams and Contactins, together leading us to posit the existence of an "immunoglobulin superfamily code" for laminar specificity (Yamagata and Sanes, 2008, 2012).

Studies in Mice

The retina has many advantages for studies of neural circuit assembly. It is accessible because it is outside of the skull. It comes with its own lens for viewing in vivo. Its cells and synapses are arranged in neat layers and thus are easy to identify. It is a complete circuit with a single controllable input (light), a single output (the optic nerve), and few if any long-range inputs. Best of all, we actually know what it does, which cannot be said for many other regions. Yet it is a bona fide part of the brain and about as complicated as other parts of the brain in terms of cellular and synaptic diversity. In a sense, it is for synaptic specificity what the NMJ was for synaptogenesis per se: a model that has all the key molecular and cellular features of, say, the cortex, but can be studied in far more satisfying detail. So, I quickly fell in love with the retina and have studied it almost exclusively over the past 15 years.

On the other hand, the chick retina does have some drawbacks: little functional information is available about it, we were not going to be able to study post-hatching stages, and it is refractory to the sort of genetic analysis that had proved to be so powerful in studies of the NMJ. The obvious solution was to transfer the retinal project from chicks to mice. Two developments made this possible. First, In-Jung Kim came to the lab as a postdoctoral fellow and undertook a heroic screen of more than 100 immunoglobulin superfamily molecules to find ones expressed in subsets of mouse RGCs. Second, once I moved (back) to Harvard in 2004, I established a collaboration with one of the leading scholars of retinal circuitry, Markus Meister.

My path back to Harvard began in 2000. Around that time, I realized that I was coming to a "now or never" point. Either I would move in the next few years or I'd soon become less marketable (I was already 51) and

would likely finish my career at Wash U I loved Wash U and loved science but remained less than enamored of St. Louis and wanted another professional challenge. The obvious solution was to do more or less the same thing somewhere else. Luckily, Harvard came calling. Neurobiology at Harvard had long been centered at the Medical School. In 2000, the dean at the time, Jeremy Knowles, called a meeting to discuss the idea of establishing a new center for neuroscience on the Cambridge (undergraduate) campus. I was part of a group invited to discuss the idea and became excited by the prospect. Over the next year, the idea of launching a center crystallized and the prospect of leading it was enticing—but Harvard had other ideas and I began to explore other options. A few years later, their exploration complete (or failed), I was offered the job and took it.

There is a long story to be told about the Center for Brain Science, but I won't tell it here. In brief, we decided to focus on trying to find out how neural circuits underlie behavior. This area was far from my own, but I was convinced it would be the most productive over the next few decades—and also one that would allow us to harness strengths in social and physical sciences that could distinguish us from medical school–based groups. We would be open to any model system (mice, fish, flies, worms…), and method (physiology, anatomy, computation, behavior…) as long as the investigator shared that overarching goal. We also realized that the tools needed to achieve the goal were insufficient, and we supported efforts to invent new and better methods. At the outset, progress was rapid but slowed down for a few reasons. First, Larry Summers, our visionary president and a major force in promoting life sciences on the Cambridge campus, put his foot in his mouth one too many times and was dismissed; his successor was far less of an advocate. Second, 2008 happened, and resources became scarce. And by the time the ship had righted itself, we were no longer (as a friendly dean explained it) the Shiny New Thing; there were now Newer New Things. Nonetheless, over a period of 10 years, we recruited a superb group of neuroscientists (e.g., Takao Hensch, Jeff Lichtman, Bence Olveczky, and Nao Uchida), who joined and helped coalesce several already on campus (e.g., Catherine Dulac, Markus Meister, and Venki Murthy), and also urged tool builders in the physical science (e.g., Adam Cohen, Xiaowei Zhuang, and Charles Lieber) to turn their talents to inventing tools for neuroscience. By the time I stepped down in 2020, the center was well established as one of two key nodes of neuroscience at Harvard, the other being the Department of Neurobiology at the Medical School.

But back to the retina. As soon as I got to Harvard, In-Jung Kim began collaborating with Yifeng Zhang, a postdoctoral fellow in Markus Meister's lab. Having completed her screen of immunoglobulin superfamily genes, In-Jung had begun generating transgenic lines to mark RGC subsets with fluorescent proteins. Yifeng learned to target fluorescent RGCs in these lines for patch clamp recording and elucidated the visual features to which

they were most sensitive. This allowed them to characterize RGC types by a combination of molecular, physiological, and structural features. They discovered several novel types, including J-RGCs and W3B RGCs, and found novel distinctions among RGCs responsive to motion in different directions (Kim et al., 2008, 2010; Kay et al., 2011a; Zhang et al., 2012). Perhaps most striking were J-RGCs, with absolutely gorgeous asymmetric dendrites that "pointed" in the direction of the motion to which they responded best; In-Jung's images now decorate a few textbooks. And in a fortuitous turn of events, the W3B RGCs turned out to express Sidekick 2; Arjun Krishnaswamy and Masa were later able to show how Sidekick shaped the response properties of this RGC type (Krishnaswamy et al., 2015).

We also dug into the cadherin superfamily, which accounts, along with immunoglobulin superfamily, for the majority of known neuronal adhesion molecules. Looking at the assembly of just the circuits that tell the brain about motion in specific directions, we found roles for six different cadherins (Duan et al., 2014, 2018). We extended the study to transcription factors that came to our attention because their genes were expressed by intriguing subsets of cells—for example, Fezf1, FoxP1, FoxP2, NeuroD6, Tbr1, and Satb1 (Kay et al., 2011b; Rousso et al., 2016; Peng et al., 2017, 2020; Liu et al., 2018)—discovering additional RGC subclasses (e.g., F-RGCs) and showing roles in circuit assembly for several of them. It was particularly satisfying that we could assess the effects of loss- and gain-of-function genetic manipulations by electrophysiological as well as morphological criteria, providing a link between circuit assembly and circuit function.

A few of these genes led us to an aspect of organization that we hadn't been looking for—the arrangement of cells and processes along the surface of the retina, or what we called lateral specificity as opposed to laminar specificity. Retinal cell types are nonrandomly arrayed along the retinal surface, a so-called mosaic arrangement that ensures coverage of all parts of the visual field by a full complement of feature detectors. Jeremy Kay isolated several retinal cell types by FACS from our GFP lines and profiled them by microarrays (this was before RNAseq was widely available). Among the genes he studied were MEGF10 and 11, two transmembrane proteins selectively expressed by a set of amacrine neurons called "starbursts" that play a key role in direction selectivity. He found that MEGF10 and its homologue, MEGF11, regulate the mosaic arrangement of starbursts, the first molecules implicated in this process for any cell type (Kay et al., 2012). Starbursts also figured prominently in studies of a remarkable clade of more than 50 tightly linked genes within the cadherin superfamily, the clustered protocadherins, which we had begun analyzing many years earlier (Wang et al., 2002). Julie Lefebvre and Dimitar Kostadinov found that they act to endow starbursts with individual identities, thereby preventing dendrites of a single starburst from synapsing with other dendrites of the same starburst (self-avoidance) while allowing them to synapse on dendrites of other, nominally identical,

starbursts (self-/non-self-discrimination) (Lefebvre et al., 2012; Kostadinov and Sanes, 2015; Ing-Esteves et al., 2018).

A Retinal Cell Atlas

Most of the cell types we studied in retina were those for which we had transgenic lines that allowed us to mark, isolate, and manipulate them. In short, we were largely restricted to cells that we already knew something about. It was clear that a full account would require a more comprehensive method for cataloguing and characterizing all retinal cell types. Over the years, based on Masa's success with Sidekicks, we had attempted transcriptomic profiling of single mouse RGCs, but the results were too noisy and the numbers too small to be useful. Our opportunity to expand the search came through a project begun with Aviv Regev at the Broad Institute and Alex Schier in the Center for Brain Science. Under the auspices of the BRAIN Initiative, the NIH had allocated funds for development of new "neurotechnologies," and we were awarded a large grant to support several groups trying to develop methods for more efficient transcriptomic characterization of large numbers of cells. The idea was that we would use the retina, for which so much ground truth was available, to validate and optimize the methods. (I had served on the planning committee for the BRAIN Initiative, but we had disbanded before grant applications were solicited, and so there was no conflict.)

The clear winner was a method called "Drop-seq," invented by Evan Macosko in Steve McCarroll's lab (Macosko et al., 2015). In this method (and two other versions invented nearly simultaneously), a microfluidic device captures single cells (or, more recently, single nuclei) in nanoliter-size droplets along with a small bead that captures the mRNA. The mRNA from thousands of cells is then converted to DNA and amplified in a single tube, yielding 100- to 1,000-fold savings in time and money compared with previous laborious one-cell-at-a-time methods. An elaborate barcoding system ensures that transcripts from a single cell can be grouped after sequencing, and computational methods are then used to divide the cells into clusters or putative cell types.

The method and its variants are so powerful and informative that they have been the main focus of the lab's work for the past six years. In a first set of experiments, Evan used Drop-seq to profile some 45,000 mouse retinal cells, which then were divided into 39 clusters. By inspecting genes selectively expressed in each cluster, and relating them to what we already knew about the retina, we could see that they all made sense and made up a low-resolution retinal cell atlas—low in the sense that many clusters contained cells of several closely related types. We then went on to enrich the most heterogeneous classes so we could obtain enough cells to finish the job, eventually ending up with what we believe to be a nearly complete atlas of

130 neuronal types plus another 10–15 non-neuronal types (Shekhar et al., 2016; Tran et al., 2019; Yan et al., 2020a). This impressive number, along with subsequent data from other brain regions, supported the assertion I'd been making for many years with limited evidence: that although the retina is easier to study than other brain areas, it is no less complex. Importantly, we checked whether types defined molecularly correspond to types defined by criteria that most neurobiologists care about—structure and function. Fortunately, at least for retina, they do.

With the atlas in hand, we have spent the past few years using it to ask biologically interesting questions. They include: How do newborn RGCs diversify into the 45 distinct types present in the adult (Shekhar et al., 2022)? Do different RGC types vary in their resilience or vulnerability to injury, and can we use those difference to improve the resilience of vulnerable types (Tran et al., 2019; Jacobi et al., 2022)? What are the cell types in the retinas of humans and nonhuman primates, and how do they differ between the peripheral retina and the fovea, which mediates our high-acuity vision (Peng et al., 2019; Yan et al., 2020b)? Can we use atlases from multiple species to learn something about the evolution and conservation of cell types (e.g., Yamagata et al., 2021; Kölsch et al., 2021). Answering these questions will take longer than my lab will be open, but fortunately, several of the key figures in these studies now have their own groups—Yirong Peng at UCLA, Karthik Shekhar at UC Berkeley, and Nick Tran at Baylor—where they each are taking this work in exciting new directions.

Sabbaticals

I am always surprised that so few tenured faculty members take sabbaticals. I have had six, each a valuable experience scientifically and an exciting adventure personally. I described the first, at Institut Pasteur, previously. Here are the others:

Cal Tech: 1993

For the first few years of our collaboration, John Merlie supervised all experiments that involved recombinant DNA, but as time passed, most of the students and postdocs in my lab incorporated molecular methods into their projects. This started to get embarrassing for me, because I had no practical experience in any of these techniques. I therefore arranged to spend several months at Cal Tech learning molecular biology, under the auspices of a Wiersma Professorship, which provided our family with a house near campus. I wanted to find a lab directed by someone senior enough to be a wise mentor but junior enough to be in town most of the time. Kai Zinn, a fly geneticist who had worked with Tom Maniatis and Corey Goodman, fulfilled both criteria—and turned out to be a patient teacher as well.

By 1993, several genes encoding homophilic neural recognition molecules (i.e., ones that bind to their own kind) important for neural connectivity had been identified, cloned, and sequenced in both flies and mice. Orthologs had been postulated, but their sequence similarity was decidedly unimpressive. My idea was to generate recombinant proteins from both species and ask whether they bound to each other. If they did, it would not only be interesting but also provide a new way to find mammalian cell recognition molecules.

With Kai's close guidance, I set out to clone extracellular binding domains, insert them into expression vectors, purify recombinant proteins, and assess binding by a phage display assay. This all worked, but I ran out of time. New Year's came, it was time to return to St. Louis, and attempts to interest students in my lab in continuing the work failed. I still think these would be experiments worth doing, although their value declined once genome sequence became available. Nonetheless, the sabbatical was a success in that I came back with enough hands-on experience to have some modest credibility in directing molecular projects in my own lab.

University of the Mediterranean at Luminy: 1999.

I met Chris Henderson during my sabbatical at the Institut Pasteur. He had been a postdoctoral fellow with Jean-Pierre Changeux, then stayed on briefly as a junior faculty member before moving south to Montpelier and then east to Luminy, a campus set close to the Mediterranean in a particularly beautiful part of Provence. Chris's interest in trophic support of motoneurons and mechanisms underlying motoneuron diseases (especially amyotrophic lateral sclerosis) overlapped mine in neuromuscular development, so we saw each other at various meetings over the years and remained friends. In thinking about a next sabbatical, the idea of learning his methods for purifying, culturing, and studying motoneurons was appealing. The location was not an insignificant factor.

The project I chose was to see if I could generate cell lines from embryonic mouse motoneurons, with the aim of eventually being able to study those from the mutants we had generated. Today, the obvious strategy would be to edit the genome of stem cells and then direct their differentiation into motoneurons, but that wasn't an option at the time, so I used a method of hybridizing embryonic motoneurons to immortalized lines. I achieved some success, and we made use of a few of the lines later, although the development of these better methods soon made mine obsolete. It was a happy time, though: I learned a lot, had the first chance to work with my own hands since Merlie's death, and absolutely loved Provence—especially the charming, small village of Cassis where we (and Chris) lived. We've gone back to that area for vacations every few years since.

Cambridge University: 2008

By 2008, the lab was largely devoted to studies of circuit formation in retina. I was in an embarrassing position not dissimilar to the one that had motivated my sabbatical at Cal Tech: I was directing a program on a topic about which I had inadequate knowledge. When the possibility arose of spending a term in Cambridge as a Visiting Fellow Commoner at Trinity College, I decided to use it as an opportunity learn about the retina. I was loosely affiliated with the conjoined laboratories of Bill Harris and Christine Holt, both of whom were leaders in the area—Bill primarily using zebrafish to study retinal lineage and Christine primarily using Xenopus to study retinotectal pathfinding. I attended their lab meetings each week but, unlike my stay at Cal Tech, I did not work in the lab. Instead, I visited with local experts (e.g., Leon Lagnado and Horace Barlow) and immersed myself in the relevant literature. As a way to enforce some discipline, I took on authorship of two substantial reviews, one with Masa Yamagata on synaptic specificity (Sanes and Yamagata, 2009) and the other with Larry Zipursky, comparing the visual systems of insects and vertebrates (Sanes and Zipursky, 2010).

Biogen: 2013

My next sabbatical was spent at Biogen, one of the world's oldest and largest biotech companies. Biogen is less than a half mile from our home in Cambridge, but it was in many ways a different world. Spyros Artavanis-Tsakonas, an eminent Drosophila geneticist with a long-time involvement in the world of biotechnology, had been recruited to be chief scientific officer a few years earlier. Biogen had a distinguished history: two of its founders, Phil Sharp and Walter Gilbert, were Nobel laureates, and another founder, Charles Weissmann, cloned interferon-alpha, initiating the company's long dominance in therapies for multiple sclerosis. However, the tradition of basic research (called "early discovery research" in biotech) had waned over the years, and Spyros's mission was to revive it. To this end, he tried to hire scientists from academia in a variety of capacities. I had become friends with Spyros, an extraordinarily gregarious individual, on an NIH study section many years earlier, so I was one of the many he called. Learning that I was not interested in moving from academia, he invited me to test the waters by spending a sabbatical semester there. It was an intriguing idea.

I began by proposing a program to use the retina as a model for testing mechanisms, targets, and therapeutics for neurological diseases. There was a lot of enthusiasm for the plan at first, but Biogen is a business, and soon their interest moved from using the retina for research to finding therapies for retinal diseases. Since then, I have served as a consultant helping to get that program launched. This has involved developing strategic plans, reviewing

smaller biotech companies that are eager to partner with or be acquired by a larger entity, and helping to hire people who actually knew what they were doing. Most recently, because of an administrative reorganization, I ended up as interim head of ophthalmology research—an appointment that was meant to last a few months but, thanks to COVID, ended up lasting over a year.

It has been both a fascinating and a frustrating experience. I had minimal previous contact with the worlds of biotech or clinical medicine, so there was a lot to learn and I enjoyed learning it. I am disappointed, however, that Biogen slowly abandoned Spyros's goal (and that of the then-CEO George Scangos) of inventing new therapies, and instead retreated to a strategy based largely on buying ideas, reagents, methods, and drugs from other companies. In short, there has been nothing to make me second-guess my decision to stick with academia.

New York University Medical Center: 2017

Steve Burden, who I mentioned earlier, had remained a good friend for some 40 years. Through him, I got to know his partner, Ruth Lehmann, who studies germ and stem cell development in Drosophila. Steve and Ruth had moved from MIT to the Skirball Institute at NYU (now, thanks to vigorous fundraising, the NYU Skirball Institute in the NYU Grossman School of Medicine at NYU Langone Health Center) many years earlier, as directors of their neuroscience and developmental biology programs, respectively; Ruth later became director of the entire Skirball Institute as well as chair of cell biology.

Steve and Ruth arranged for Susan and me to spend the fall of 2017 at NYU, and Ruth even managed to get us a studio apartment in a building on the health sciences campus. It ended up being a semester of very long weekends: we'd take the train from Boston to New York on Thursday evening, and return to Boston of Tuesday evening. Thus, I could spend Wednesday and Thursday in my lab at Harvard (with Susan spending those days at the legal services center where she was then working), work from New York the other three weekdays, and explore the city on the weekends.

My main neuroscience-related activity in New York was to revise chapters for the next edition of *Principles of Neural Science*. The first edition of this textbook was written by Eric Kandel (see Volume 9) and James Schwartz in 1980. Tom Jessell, who joined the team for the third edition, asked me to coauthor the section on neural development for the fourth edition, which appeared in 2000. This was a massive task, because the field had advanced radically over the preceding decade, and we undertook to completely reimagine and rewrite the section. We wrote what was essentially a short book inside a long book. In fact, we contacted the publisher to ask whether they would consider letting us expand the section modestly (dividing each jam-packed somewhat telegraphic chapter into two slightly

more discursive ones) and turn it into a stand-alone volume. They refused. By the time of the fifth edition (2012), Tom had taken on leadership responsibilities at Columbia and what had been a shared burden fell mostly on me. For the sixth edition, Tom faced serious health and other problems, so I ended up doing it entirely myself. By the time the edition finally appeared in 2021, Tom had passed away. With his death, that of Schwartz much earlier, that of the artistic director, Sarah Mack, in 2020, and the decreased involvement of Eric Kandel, my guess is that the sixth edition will be the last.

Family

The reader may wonder whether I abandoned my family during these adventures. In fact, things worked out well. Susan knew she was pregnant before we went to Paris and enjoyed the opportunity to slow down and appreciate the process. Her employer in St. Louis (Planned Parenthood) gave her a leave of absence; given their name, how could they not? Jesse, our son, was born in April and we were able to appreciate his first few months and host his grandparents before returning to "real life."

Seven years later, Susan was able to use our semester in Cal Tech as a pretext to extricate herself from an administrative job (not at Planned Parenthood) that she had outgrown and accept a new position. Jesse, now joined by Milla, were 7 and 5. We enrolled them in the local school, in which most of the students were working class and foreign born. Their exposure to people they would not have seen in their excellent but liberal/yuppie St. Louis school was a valuable experience.

By the time we went to Cassis, Susan had decided to go to law school. She studied hard, aced the LSATs, and was admitted. Time in Cassis gave her a chance to take a breather, get ready for the rigorous three years ahead, and leave her current position sooner than would have been politic otherwise. As to school, we found out what our children would need to do to return without penalty and made sure they did it. We had asked them to try out the local schools but gave them the option to stay home if things didn't work out. In fact, both thrived, made friends, became fairly fluent in French, and came home with the desire to visit again, which both have done.

Final Thoughts

Mentors and Collaborators

A majority of the authors in this history series owe much of their success to the students and fellows who worked in their laboratories and actually carried out the experiments for which their mentors got the credit. I am certainly in that category. I've tried to acknowledge their contributions

to the work I described, and I remain proud by proxy of their subsequent achievements. It would be impolitic to name those to whom I felt closest or who went on to greatest glory—but they know who they are.

What I've also tried to do is emphasize the importance of mentors and collaborators, perhaps less generally acknowledged. I had mentors every step of the way: Robert Guthrie in high school, Paul Greengard in college, John Hildebrand in graduate school, Jack McMahan and Zach Hall during postdoctoral study, and Dale Purves when I was a junior faculty member. I don't know where I'd be without their wisdom, guidance, and forbearance—but I'm sure I would not be writing this chapter.

Likewise, as important as students to me (heretical though it may seem) were true collaborators: faculty in other laboratories. Here, I'm thinking of those with whom there was a relationship that extended beyond a single project or paper: Ann Marie Craig, John Lawrence, Jeff Lichtman, John Majors, John Merlie, Alan Pearlman, and Rachel Wong at Wash U; Zhigang He, Markus Meister, Aviv Regev, and Larry Zipursky since moving to Harvard. Of these, I'd single out John Merlie, Jeff Lichtman, and Markus Meister as having particularly profound effects on the direction of our work.

Style

It is the height of hubris to talk about a scientific style, but as I look back, I see that we have in most cases tried to answer a question rather than exploit a specific method. In service of answers, I was perfectly happy to play histologist to Merlie the molecular biologist, molecular biologist to Lichtman the histologist, and geneticist to Meister the physiologist. The advantage of this approach is obvious, but it does have disadvantages. We never became true experts in any single method, nor did we have simple projects that would allow undergraduates or beginning graduate students to "turn the crank." We were never able to knock at the gates of Hershey Heaven, the vision of scientific joy enunciated by the pioneer molecular biologist, Alfred Hershey: "to have one experiment that works and keep doing it all the time." And I do think failure is more common with this approach than with a methods-driven one—see how many times I've used that word or its relatives.

A few months ago, I heard a related idea put forward by Ardem Patapoutian, who won the Nobel Prize in 2021. At the end of a (virtual) seminar I attended, he had a single "advice for young scientists" slide. It showed a Venn diagram in which one circle labeled "big questions" overlapped only slightly with another circle labeled "enabling technology." He advised ambitious neuroscientists to focus their attention on the overlap. This captured my view perfectly. There are some questions I would love to have addressed, such as what causes schizophrenia or what is consciousness—but there was no way to approach them in a way I thought was rigorous. On the other side, there were no end of questions we could have answered with the methods

we had—but I couldn't make myself care about many of them. I tried to stick to the overlap.

Next Step

Some of my friends in the world of science wanted to work until their last day on earth—notably John Merlie and Paul Greengard, both of whom got their wish. Not me. Neurobiology research has always been the most fascinating activity I could think of, but never the only one. I believe that for most scientists, it's a good idea to get out of the way at some point, making room for younger people with more energy, more passion, new technological expertise, and better ideas. When I began telling friends several years ago that I planned to phase out my lab by my early seventies, the invariable response was, but what will you do? The answer is, I don't know. Having viewed myself primarily as a neurobiologist for more than a half-century, I'm curious to find out what it feels like to not be a neurobiologist. This is scary to be sure, but quite exciting.

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