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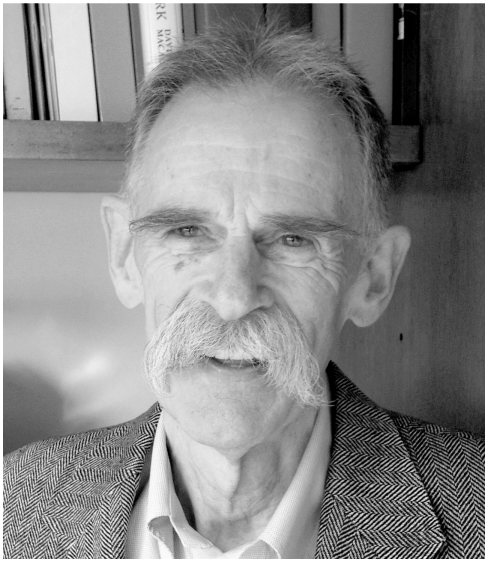

Nicholas C. Spitzer

Born:

New York, New York
November 8, 1942

Education:

Harvard College, BA (Biology, 1964)
Harvard Medical School (1964–1966)
Harvard University, PhD (Neurobiology, 1969)

Appointments:

Fellow, Harvard Medical School (1969–1970)
Fellow, University College London (1970–1972)
Assistant to Distinguished Professor, University of California San Diego (1972–present)
Chair, Department of Biology (1988–1990); Co-Chair (1992–1993)
Chair, Academic Senate (1994–1995)
Founding Chair, Neurobiology Department, Division of Biological Sciences (1999–2003)
Founding Co-Director, Director, Kavli Institute for Brain and Mind (2004–2020)
Founding Editor-in-Chief, BrainFacts.org (2011–2014)

Honors and Awards (Selected):

Alexander Forbes Lectures, Grass Foundation, MBL (1998)
Trustee, The Grass Foundation (1998–2002)
Elected Councilor, Society for Neuroscience (2000–2004)
Elected Fellow, American Association for the Advancement of Science (2001)
Elected, American Academy of Arts and Sciences (2002)
Wiersma Visiting Professor of Neuroscience, California Institute of Technology (2006)
Elected, National Academy of Sciences, USA (2013)
Atkinson Family Endowed Chair (2016–present)
Kuffler Lectures, University of California, San Diego (2017)
Karl Spencer Lashley Award (2022)

Nick Spitzer carried out a sustained line of research originating in studies of the development of neuronal excitability. This led to the discovery, in the embryonic amphibian nervous system, of calcium-dependent action potentials that generate transient elevations of intracellular calcium before the action potentials achieve classical sodium-dependence. He found that changes in this calcium-dependent electrical activity cause neurotransmitter switching, which is associated with matching changes in postsynaptic receptors. He subsequently discovered that changes in electrical activity also drive transmitter switching and receptor matching in the adult mammalian brain. Transmitter switching often switches the sign of the synapse and leads to changes in behavior that can be detrimental or beneficial. These findings identified a novel form of plasticity, distinct from changes in synaptic weight and synapse number, and overturned the long-held view that the choice of transmitter is irreversibly specified by cell identity at an early stage of development.

Nicholas C. Spitzer

Parents

I was born into an academic family with high expectations of achievement, shortly after the United States entered World War II. In addition to the chromosomes, there was a culture of accomplishment. I am grateful for both, but have always wondered how my career would have turned out with a different starting point. *Finis origine pendet*—the end hangs on the beginning—so I shall start with a description of the family into which I arrived.

My father, Lyman Spitzer Jr., was an astrophysicist who in 1946, when I was four, published a report for the Rand Corporation pointing out the benefits of space astronomy and the value of an orbiting telescope. He would go on to develop suborbital telescopes and promote the realization of the Hubble Space Telescope. These and other achievements were later recognized in many ways, including the National Medal of Science, the Crafoord Prize, and posthumously when the last of the four great orbiting telescopes was named the Spitzer Space Telescope. At the time I was born, he was working in the Empire State Building on development of sonar to enhance submarine detection for the Navy. He was younger than his two sisters and the older of two sons of a Toledo Ohio businessman who ran a paper box factory. He had won laurels in boarding school at Andover Academy and in college at Yale, received his PhD at Princeton, and was on leave from his faculty position at Yale. At the age of 33, he was appointed professor and chair of the Department of Astronomy at Princeton and director of the University Observatory.

My mother, Doreen Canaday Spitzer, was the archeologist daughter of a Toledo industrialist at the Willys Overland Company who was known as the father of the Jeep. She attended boarding school at Dongan Hall on Long Island and college at Bryn Mawr, her major in archeology inspired by her mother's devotion to the classics. After graduation, she spent three years as a student at the American School for Classical Studies at Athens, working on excavations in Corinth, Greece. This launched a lifelong love of Greek language, culture, and the arts. She later returned to Greece many times and served as a trustee of the school for 18 years. She took all four of her children and later 10 of her 13 grandchildren on individual trips "to the old country." Being the eldest, I had the privilege of being the first. My mother was also a sparkling hostess at dinner parties in Princeton for a steady stream of visiting astronomers. In early years, my sisters and I watched them come and go but ate supper by ourselves in the kitchen. In later years, we were welcomed at the dining room table, fascinated by the conversations.

Lyman and Doreen met each other at an early age, as the two families knew each other socially in Toledo. They were mutually attracted and enjoyed dancing together but decided to pursue their different objectives for a while and stay in touch. They met again in Switzerland when the Spitzers invited the Canadays to visit them at the Chateau Gumligen bei Bern. Doreen and Lyman played tennis, recited poetry, danced, and climbed the Stockhorn. Their wedding in 1940 led to a happy and mutually supportive marriage of 57 years, as Doreen recounted in her memoir, *As Long As Ye Both Shall Live*.

My father studied interstellar matter and star formation from both theoretical and observational perspectives. He was known for an unusual ability to visualize physical processes, examining them in his mind and then checking conclusions mathematically. My mother delved into the origins of the culture she loved so much. My parents' interests in these evolving and changing processes likely contributed to my later interest in the development of the nervous system.

Childhood

In connection with my father's war work, our family moved to Washington, D.C., where my sister Dionis was born in 1945. We subsequently moved back to live in New Haven, Connecticut, before moving to Princeton in 1947. The Observatory on Prospect Avenue adjacent to the campus was connected to a three-story Victorian house with stairs equipped with bannisters that we loved to straddle and slide down. My sisters Sarah Lutetia and Lydia were born in 1950 and 1954, and Dionis and I graduated to bedrooms on the third floor of the house. We enjoyed climbing on the peaked, slate-tiled roof with its wide gutters and in the towering maple and beech trees in the yard.

My father installed a chairlift in the yard on a rope that ran from a low point on one tree to a high point on another; we took turns pulling each other up with a tow rope, letting go the rope and watching the rider come careening down in high glee. He also dug a small swimming pool in the yard that was later enlarged in excavation by the faculty and graduate students of the department in a single afternoon at one of the annual Observatory picnics. My mother built a playhouse nearby from the large shipping crate in which our grand piano arrived. She also converted a back room of the house into a playroom with a curtain that fostered impromptu theatrical performances. She enjoyed gardening and taught me how to separate bulblets and plant out daffodil and tulip bulbs.

The house was situated on the same street as the University eating clubs and saw a steady flow of undergraduates along the sidewalks on which we roller skated. When there was significant snowfall in winter (and snow days with no school), we went skiing on nearby country slopes, climbing up for each descent. When our neighbor Don Griffin brought out his horse and

sleigh on Prospect Avenue, we rode in the sleigh or skijored behind. Both my parents loved to ski and from an early age took us on week-long ski vacations from school every year at Lake Placid, New York, or Stowe, Vermont. I continue to enjoy both downhill and cross-country skiing with family and friends. I have faint memories of my attendance at Miss Mason's Nursery School and Miss Fine's School, followed by stronger recollections of the Nassau Street grade school that was a short walk from home. My parents were ardent Francophiles (my father took all his sabbatical leaves in Paris), so my mother arranged for a series of French undergraduates to provide brief afternoon tutorials for me and my sisters in conjugating *avoir* and *être* and learning a modest vocabulary. These were followed by a demonstrably French dinner at which we all had to speak French. After dinner she played the piano and we gathered round to sing French songs.

When my father took his position at Princeton, he had arranged for a six-month leave of absence every other year to work at Caltech where he could make observations with the large telescopes on Mt. Wilson and have opportunities for collaboration. Some years we took a train to Chicago and another one on to Los Angeles, a three-day trip through spectacular western scenery. Other years we took the train to Chicago and drove the rest of the way, camping out and visiting National Parks. We lived in Pasadena in a series of rented houses near Caltech and were enrolled in school. We got out on hikes in the San Gabriel Mountains along old trolley lines. We stayed in the Kapteyn Cottage when my father was observing with the 100-inch telescope that Hubble had used to make observations of galaxies beyond our own and obtain evidence for an expanding universe. My father's war work had led to friendship with Roger Revelle at the Scripps Institution of Oceanography; we visited the Revelles in La Jolla and our families went camping together in the desert. The palm trees, bougainvillea, and sagebrush of southern California combined with camping and hiking trips in the Sierra Nevada mountains and in Yosemite, Sequoia, and Kings Canyon National Parks produced many warm memories. Years later, these experiences predisposed me to be enthusiastic about a faculty position at the University of California, San Diego (UC San Diego).

My father greatly enjoyed planning family trips, as had his father before him. One of these saw us sailing on the Isle de France ocean liner from New York to Le Havre in 1955. Dionis and I were introduced to the many sights of Paris and then traveled to Zermatt in Switzerland with our parents for an introduction to serious climbing with guides. We enjoyed climbing peaks on both rock and snow, but were too young to join our parents on their ascent of the Matterhorn. When my father afterward participated in a meeting of the International Astronomical Society in Dublin, Ireland, my mother, Dionis, and I cycled around the countryside exploring and climbing in the ruins of old castles. My two younger sisters stayed at home with a good family friend.

After completing fifth grade at the Nassau Street school, I progressed to the Quarry Street school for the sixth through eighth grades. I did well in all subjects and founded a student newspaper (*Teen Topics*). However, my behavior in school was rambunctious, and I was sent to the principal's office on more than one occasion for disruptive behavior in class. I was caught shoplifting in Woolworth's store and entering locked university buildings through open windows—activities that reflected my teenage testing of limits—what could I get away with? My parents sensed that I was not being sufficiently challenged academically and enrolled me at Andover Academy for high school. It was a perfect move.

Education

My four years at Andover were transformative. Academic demands were challenging and I learned how to focus my efforts. I enjoyed Mr. Maynard's Math 2x-4x, which covered three years of math in two years, and Dr. Hammond's Science Honors course. With Dr. Gillingham, I learned Latin and read Caesar, Virgil, Horace, and Ovid. Monsieur Gilbert taught me French by *la méthode direct* (he never spoke in English). Emory Basford was a stern but forgiving task master in English, and I remember memorizing long soliloquies in Shakespeare's *Richard II* (popularly referred to as *Dick the Deuce*). I played club-level sports, rowing crew or running cross-country in the fall, skiing in the winter, and playing lacrosse in the spring. I enjoyed my theatrical roles as King Claudius in *Hamlet* and the sinister Jonathan in *Arsenic and Old Lace*. I have always been grateful for the breadth and rigor of my Andover education, which prepared me to enter Harvard in 1960 as a sophomore with advanced standing.

I declared my major in physics and enrolled in math and physics courses that I soon realized were over my head. I also spent a great deal of time dating a classmate at Radcliffe whom I had met the previous summer in the Experiment in International Living in France. I was encouraged to try out for the fencing team and the épée became my weapon. I enjoyed the regular workouts and the strategies for achieving a touch against opponents, going on to become team captain my senior year. However, my grades were so poor at the end of my first year that I received a letter from the dean suggesting that I might like to take a year's leave of absence. I spent the summer in school at UC Berkeley, taking a course in English literature and another in Italian, with time off to go climbing in Yosemite. My second year at Harvard, I built on my facility with languages and pendulumed my major from physics to Slavic and linguistics, taking intensive courses in these subjects.

Fortunately, I also enrolled that year in George Wald's new introductory biology course, Natural Sciences 5. Wald was a Nobel laureate for his discovery of the role of vitamin A in forming the three color pigments and showing that color blindness is caused when one of the pigments is absent. He

was a compelling lecturer, holding the entire class in rapt attention, starting with prebiotic synthesis at the beginning of the semester and concluding with animal behavior at the end. I found his lectures on the nervous system inspiring, and learned later that he had persuaded half a dozen other students that year to take up careers as neuroscientists. I switched my major to biology and approached John Dowling (Volume 4), who had worked with Wald and was now a member of the faculty, to ask if I could work in his lab.

I was delighted when John took me on in the fall of 1962. He had just demonstrated the existence of rapid dark adaptation in the albino rat that did not have a photochemical basis—an apparently neural process. He suggested that I study the horseshoe crab, *Limulus*, to see if it had a similar component to the visual adaptation of its compound eyes. If it did, the simplicity of neural processes beyond the receptors would raise the possibility that rapid dark adaptation is a property of the receptor itself. His enthusiasm was contagious. I drove to Woods Hole, brought animals back to the Bio Labs on Divinity Avenue, and maintained them in seawater in a cold room. I spent many happy hours recording from single nerve fibers dissected from the optic nerve bundle of young crabs. For work the following summer, I had a National Science Foundation (NSF) fellowship of $600 that was just sufficient to rent a room in Cambridge and support my steady diet of whole wheat bread and peanut butter. I supplemented this occasionally with bullfrog legs from Wald's freezer where, after removing the frogs' retinas, he had stored them for his later consumption. Fortunately, I had graduated and left the lab by the time Wald returned, but I learned later that he was greatly annoyed by the loss.

My results were compatible with the existence of a neural component to dark adaptation. I loved this research. I found electrophysiology intensely satisfying: One asks the question and the data become available immediately. Change an experimental parameter, new data appear. This experience launched my career. Discussions with John both in his lab and during picnics on the Boston North Shore directly shaped the next phase of my education. He had gone to medical school at Harvard and thought that he had been more broadly educated than he would have been in graduate school, where prompt specialization and narrowing of focus is emphasized. I found this persuasive and matriculated in the fall of 1964 at the Harvard Medical School.

The entering class was told that a substantial fraction of everything we would be taught would prove incorrect, but the identity of that fraction was unknown so that it was essential to learn all of it. In the first two years, the rapid pace included gross anatomy, biochemistry, and physiology, as well as parasitology and pathology courses, all requiring hours of study. The latter courses introduced me to subjects with which I was wholly unfamiliar and contributed to my later interest in scientific problems with clinical relevance. The block of lectures on the nervous system were particularly good

and especially interesting for their clarity and rigor. They were presented by faculty in Stephen Kuffler's neurobiology unit, at that time a component of Otto Krayer's Pharmacology Department. In the second year, we were given white coats and introduced to taking histories on hospital wards. This tipped the scale for me, as I soon realized that there is a routine and even repetitive quality to medicine because patients often have the same disorders. I was sufficiently self-aware to recognize that continuity of this experience would not be sustainable.

With the prospect of two more years of study focused increasingly on clinical aspects of medicine, I gave my college senior honors thesis to Steve Kuffler in support of my application to become a graduate student in the neurobiology unit. I was very glad to be accepted and supported by a predoctoral fellowship from the NIH. The extraordinary faculty included David Potter, Ed Furshpan, Ed Kravitz (Volume 4), David Hubel (Volume 1), and Torsten Wiesel. Jim Hudspeth, David Van Essen (Volume 9) and Eric Frank joined me in this class of graduate students. Demanding seminar courses taught by the faculty, based on original papers, were intensely stimulating. David Potter became my adviser and got me started with a cathode follower amplifier, making intracellular recordings with sharp electrodes and replicating published findings. As postdoctoral fellows, Dave and Ed Furshpan had discovered the electrical synapse in the crayfish nervous system, and they were pursuing their joint interest in electrical coupling among electrically inexcitable cells. Dave suggested the rat seminiferous tubule as an experimental preparation because of the potential role of somatic supporting cells in coordinating synchrony of meiosis by large numbers of germinal cells. I was able to be quite independent, and I became interested in a similar arrangement in the anther of the lily bud, which could be conveniently investigated once a year in the spring. Short-range interactions between cellular elements might be afforded by gap junctions in the seminiferous epithelium and by plasmodesmata in the anther, directly connecting the interior of one cell to that of another. In both of these preparations, my recordings of electrical coupling and observation of the spread of intracellular tracers demonstrated interaction between cell types not previously known to be joined by appropriate membrane junctions. However, I did not obtain evidence for a role for these connections in controlling or metabolically supporting synchronous meiosis. I received my PhD in 1969 and published my first paper on the results from the anther of the lily (Spitzer, 1970). My unpublished results of coupling in the rat seminiferous tubules later received published support in the form of electron microscopic images of gap junctions (Pelletier & Byers, 1992, *Microscopy Research Technique,* 20, 3–33).

Steve's neurobiology unit had become the Neurobiology Department by the time I received my PhD. Steve ran it like a family, warmly engaged in everyone's work and life. He led by example, scientifically and personally.

At seminars in the lunchroom, he illustrated how one could be penetratingly critical and also exhibit an extraordinary sense of humor, sometimes simultaneously. His influence on members of the department in those times is captured in a wonderful monograph (*STEVE, Remembrances of Stephen W. Kuffler*; McMahan, 1990).

Postdoctoral Years and Future Planning

Jack McMahan was working with Steve and pioneering the use of Nomarski interference contrast optics for visualization of neurons *in situ*. I approached him to ask if I could do a brief postdoc with him. I had spent the summer between my two years in medical school in the lab of J. David Robertson, learning electron microscopy (EM). Robertson's technician, Janet Lamborghini, taught me how to cut thin sections and capture exquisite EM images. The experience not only gave me a deep appreciation of the power and beauty of anatomy, but also introduced me to my future wife (married in 1967). My studies with Jack enhanced my appreciation of the importance of anatomy when we developed the frog cutaneous pectoris muscle as a preparation for direct visualization of single nerve terminals for physiological studies.

Steve was a good friend of Bernard Katz (Volume 1) from their time together in Sydney, and there was an academic pipeline bidirectionally between Steve's Department and Katz's Biophysics Unit at University College London. I was impressed by the way Bernard Katz and Ricardo Miledi thought about solving scientific problems and asked Steve if I might pursue further postdoctoral work there. In response to my letter of inquiry to Katz, I soon received a letter of welcome in return. The next two years were exhilarating. Dale Purves (Volume 11), Bert Sakmann, Bill Betz, Jean Rosenthal, Terje Lomo (Volume 7), and Bob Zucker contributed to the lively discussions of research (or cricket) in the tearoom at 11 a.m. and 4 p.m., in which Katz and Miledi occasionally participated. I worked under the supervision of Ricardo, studying the effect of botulinum toxin on synaptic transmission at the amphibian neuromuscular junction and collaborating with Mollie Holman during her sabbatical visit from Monash University. Ricardo was an extraordinarily creative investigator and I watched him closely in order to absorb as much of his talent as possible. Living in London on a NATO NSF postdoctoral fellowship enabled enjoyment of West End theater, music on the South Bank, side trips to notable sites in the countryside and travels in Europe.

As my second year in the Biophysics Unit moved along, I started to think about what to do next. I believed that the careful choice of the problem on which to begin work in my own lab would be essential and could define the arc of my career. It was critical to select a problem in which I had a deep interest so that I would work tirelessly to address it. I wanted to go in untraveled directions and pick a problem unidentified by others

or that had received little attention. A less crowded field would provide more opportunities for significant discovery, and it would be important to embrace risk. I wanted to select the largest problem on which I thought I could make serious headway in a reasonable time. It would also be useful to pick a problem that appeared to be fundamental and thus of broad significance. I grew up intellectually in the heyday of molecular biology in the 1950s and 1960s. Fundamental questions about DNA structure and replication, coding of information in DNA, and operation of the Central Dogma had been answered. These discoveries were inspiring, and I was looking for similarly foundational questions to address.

Neuronal development was endlessly fascinating to me although I had had no practical experience in the field. The interface of electrophysiology with developmental biology was relatively unexplored. I boiled down my choice to either the development of electrical excitability or the development of specific neuronal connections. Both action potentials and brain wiring are crucial to brain function. Studies of axon pathfinding and connectivity were already underway by Michael Gaze, Marcus Jacobson, and others. Much work focused on development of retinotectal connections that Roger Sperry's incisive studies had drawn to attention. However, the use of genetically tractable models for developmental studies was in its infancy. Ed Furshpan and Dave Potter had led a graduate seminar discussing the famous five Hodgkin (Volume 1) and Huxley (Volume 4) 1952 papers in the *Journal of Physiology*. Like many others, I was captivated by their findings. How did the action potential develop during embryogenesis? I wanted to find out.

In my graduate work, I had learned how to make intracellular recordings from small cells in novel preparations. My knowledge of Kuffler's pathbreaking research made me aware of the importance of selecting the appropriate model system with which to answer a question. In my postdoctoral studies, I was sensitized to the advantages of an amphibian model. The available literature led me to focus on the primary sensory Rohon-Beard neurons of the *Xenopus* embryo. These neurons were first described by Freud during his early career as a neuroanatomist. Their names came from James Victor Rohon, in Germany, and John Beard, in England, who studied them later. I was to fall in love with them, as they kept yielding up secrets about neuronal development. The location of their 20-μm diameter cell bodies on the dorsal surface of the neural tube would facilitate recordings. Anne Warner gave me some embryos on which to try dissections and impalements and that went well. My first goal would be to chart the ontogeny of the neuronal action potential.

Starting My Lab at UC San Diego

I flew to the United States for a whirlwind visit to five universities and found UC San Diego particularly attractive. Twelve years earlier, Roger Revelle

had recruited outstanding faculty with whom to launch the University, and its cross-disciplinary spirit and ambition were appealing. I accepted the offer and arrived in January 1972. My startup package included a laboratory in Bonner Hall, two offices, $25,000 and several used microscopes. Janet Lamborghini was my first postdoc and helped me set up the lab. After futile efforts to capture the elusive *Xenopus* in the storm drains along the freeways, we ordered adult animals to begin generating embryos. Sharp, high-resistance microelectrodes were pulled for physiological recordings, and fixatives and microtomes were prepared for anatomical studies.

It was exciting to make the first successful impalements of Rohon-Beard neurons in the embryos and in the premetamorphic tadpoles, although success came only once or twice a week in the beginning. To provide a thorough grounding in the membrane properties of these neurons, I began by studying their resting potential, which proved interesting in its own right. It sat at –88 mV in the presence of 3 mM external potassium chloride (KCl), like the resting potential of glia (Kuffler et al., 1966, *Journal of Neurophysiology*, 29, 768–787). This value was more negative than most intracellular recordings of neuronal resting potentials known at the time, but followed the prediction of the Nernst equation for a K^+-selective membrane. Stimulation of the anterior end of the spinal cord elicited antidromic action potentials in Rohon-Beard neurons, followed by slower depolarizations. These depolarizations could be attributed to accumulation of extracellular K^+ from the action potentials of neighboring axons, as earlier demonstrated for glia (Orkand et al., 1966, *Journal of Neurophysiology*, 29, 788–806). I suggested that in intact animals, these depolarizations of the membrane potential should decrease the distance to threshold so that a smaller generator potential would be effective in eliciting an action potential (Spitzer, 1976).

The Development of Excitability: Intracellular Recordings

I was particularly eager to investigate the appearance of the action potential. My recordings from Rohon-Beard neurons at the tadpole stage had shown that the action potential was brief in duration and conventional in waveform. Does it spring into existence fully formed, like Athena from the head of Zeus? Progress with the embryos was slow because dissections were often destructive and because visualization of the neurons viewed through a 40x water immersion lens was hindered by intracellular inclusions of yolk and lipid. Fortunately, Paola Baccaglini, my first graduate student, joined me in this work. We both felt a great urgency for success and worked late into most evenings. At the neurula stage, when the neural tube had just closed, no action potential was detected in response to a depolarizing current pulse. However, a hyperpolarizing current pulse yielded an inflection in the voltage trajectory at the conclusion of the pulse, suggestive of anode break excitation. Wonderfully, depolarization of Rohon-Beard neurons in embryos

only a few hours older, at early tailbud stages, produced all-or-none, overshooting action potentials that ranged in duration from as long as 500 milliseconds (msec) to as short as 50 msec. The use of ion substitutions and pharmacological blockers led us to the conclusion that most of the inward current was carried by Ca^{2+}. Calculation suggested that the Ca^{2+} influx could raise the intracellular Ca^{2+} concentration from 10^{-7} to 10^{-5} M, sufficient to influence later neuronal differentiation. When embryos were a few hours older, at late tailbud stages, the action potential acquired a new, rapidly rising component. This was followed by a slower component that created a shoulder on the repolarizing phase. The peak proved to be Na^{+}-dependent and the shoulder was Ca^{2+}-dependent. The overall duration now ranged from 20 msec to 4 msec. Then, by early tadpole stages, the action potential was 1 msec in duration and wholly Na^{+}-dependent, as in the mature animal (although Ca^{2+}-dependent action potentials could still be elicited when K^{+} currents were blocked). These findings demonstrated the sequence in which neuronal excitability developed in the early stages of maturation (Spitzer & Baccaglini, 1976; Baccaglini & Spitzer, 1977).

Recognizing that dissociated cell cultures would be useful for extending the analysis of differentiation, we modified procedures of others so we could track the development of neurons from cells that had been dissociated from the neural plate of embryos before the onset of their excitability. It was gratifying to discover that the somatic action potential of Rohon-Beard neurons, motor neurons, and interneurons all matured *in vitro* in a similar sequence and with a time course similar to what we had observed for Rohon-Beard neurons *in vivo* (Spitzer & Lamborghini, 1976). In addition, finding this same sequence of maturation in cultures of single neurons demonstrated that the process is cell autonomous (Henderson & Spitzer, 1986). The same sequence of maturation of action potentials, from Ca^{2+}- to Na^{+}-dependence, was later observed in neurons from embryos of chick and mouse (Spitzer, 1985).

Anatomical studies of the birth of Rohon-Beard neurons, their sequestration of intracellular Ca^{2+}, their transmitter uptake and the death of the neurons were carried out in the following years (Lamborghini et al., 1979; Lamborghini, 1980, 1985, 1987). Following Steve Kuffler's practice, I added my name to publications only when I had participated in the experiments, though I was wistful when three of the papers from the lab appeared in *Nature*. Still, I believed it was the right thing to do, and it does not seem to have hindered my career. I continued this practice for 22 years until the lab grew larger, with many projects underway simultaneously, so that I needed to leave the joys of doing the experiments to my colleagues.

In 1977, Corey Goodman (Volume 12) visited and made a persuasive case for studying the development of embryonic grasshopper neurons. He subsequently joined the lab as a postdoctoral fellow. The dorsal unpaired median (DUM) neurons of grasshoppers, like the Rohon-Beard neurons

of *Xenopus*, were identifiable, such that the same cells could be examined from animal to animal. Simple dissections allowed both classes of neurons to be visualized and studied *in vivo* early during embryogenesis, and their large size expedited intracellular recordings. What aspects of functional and anatomical development of Rohon-Beard neurons were conserved in the development of DUM neurons? A grasshopper breeding colony was quickly set up in the basement of Bonner Hall. We mastered the dissections of segmental ganglia in embryos at different stages of development and investigated the anatomical, biochemical, and electrophysiological properties of DUM neurons from birth to maturation. The development of axon projections, their central arborizations and the appearance of their transmitter, octopamine, were characterized first (Goodman et al., 1979). We also examined the development of chemosensitivity to neurotransmitters, the onset of electrical excitability, and the loss of extensive electrical and dye coupling to other cells that is frequently observed during cell-specific differentiation (Goodman & Spitzer, 1979, 1981a, 1981b; Goodman et al., 1980). The earliest action potentials were elicited from neurites about the time of electrical uncoupling and were Na^+-dependent. Action potentials appeared shortly afterward in the soma and were dependent on both Na^+ and Ca^{2+}. I was early to bed and early to rise during those years, while Corey rolled in later and worked into the evening. This arrangement provided the opportunity to carry out my experiments on *Xenopus* in the morning, investigating the development of chemosensitivity and electrical uncoupling of Rohon-Beard neurons. Corey and I worked together on grasshopper in the afternoon. We concluded that the developmental trajectories of Rohon-Beard and DUM neurons were similar for aspects of development known at the time. Later, we discovered another similarity. Transmitter expression in Rohon-Beard neurons starts at the stage of development when axon outgrowth begins (Root et al., 2008; Jacobson & Huang, 1985, *Developmental Biology*, 110, 102–113), just as we had found for DUM neurons.

After completing a two-year postdoc, Corey joined the faculty at Stanford, and I returned to full-time work with Rohon-Beard neurons. I had found they were electrically coupled before the time when Ca^{2+}-dependent action potentials could be recorded. The coupling was voltage-dependent, and sustained depolarization or hyperpolarization of one neuron reversibly uncoupled that neuron from others, as previously reported for amphibian blastomeres. I was intrigued by the voltage dependence of this process. Reduction of intracellular pH eliminated the voltage dependence but left the neurons in a coupled state. When Ca^{2+}-dependent action potentials appeared, the impulse in one neuron could uncouple it from others. At early stages of development, Rohon-Beard cells were coupled along and between two rows on either side of the dorsal spinal cord. These neurons became permanently uncoupled around the time that the prominent Na^+

current appeared in the action potential, while other cells remained coupled (Spitzer, 1982). The role of voltage-dependent uncoupling was unclear, but I supposed it might serve to isolate neurons from one another, so that they could begin to develop independently.

Postdoc John Bixby and I completed the study of developing chemosensitivity in Rohon-Beard neurons, finding that responses to GABA first appeared at late tailbud stages. The responses had a reversal potential of –30 mV, which appeared to depend on Na^+ and K^+ instead of Cl^- and did not change during development, but were blocked by bicuculline or picrotoxin. Later research indicated that elevation of the intracellular concentration of Cl^- by a Na^+- and K^+-dependent Cl^- transport mechanism (likely NKCC1) underlies the Cl^--dependent depolarization by GABA receptors in these neurons (Rohrbough & Spitzer, 1996). Surprisingly, both young and mature neurons appeared to be insensitive to glutamate, acetylcholine, or five other transmitters (Bixby & Spitzer, 1982). Responses to GABA appeared at about the same time as the appearance of the Na^+ current in the action potential and contemporaneous with electrical uncoupling. In addition, study of the appearance and development of transmitter sensitivity in cultures of *Xenopus* spinal neurons demonstrated the presence of two populations of cells. One population had the chemosensitivity profile of Rohon-Beard neurons, depolarized by GABA and insensitive to glutamate. The other population had the profile of motor neurons and interneurons. They were depolarized by glutamate and were hyperpolarized by GABA and glycine, with a reversal potential of –59 mV (Bixby & Spitzer, 1984a).

In the meantime, we demonstrated a requirement for RNA and protein synthesis for maturation of the action potential, using our cell culture system. Early inhibition of transcription with actinomycin D, or inhibition of protein synthesis with cycloheximide or puromycin, blocked the appearance of brief Na^+-dependent action potentials such that they remained long in duration and Ca^{2+}-dependent. Application of the inhibitors at later times in development allowed action potentials to develop normally (O'Dowd, 1983; Blair, 1983).

The Development of Excitability: Voltage Clamp Studies

The next step was to make voltage-clamp recordings of the ionic currents underlying the action potential to quantify their changing contributions during development. Patch electrodes had recently been perfected, and these seemed ideal for voltage-clamp recordings from cultured cells (Hamill et al., 1981, *Pflugers Archiv*, 391, 85–100). As part of her graduate training, Diane O'Dowd took a Cold Spring Harbor course to learn how to voltage clamp excitable cells and set up our first patch-clamp rig when she got back to the lab. Angie Ribera joined me for her second postdoc and became an

invaluable colleague. In short order, we recorded isolated Ca^{2+}currents, Na^+ currents, and both voltage- and Ca^{2+}-dependent K^+ currents from young neurons and mature neurons. The high-voltage-activated Ca^{2+} current (I_{Ca}) showed only a small increase in the extent of inactivation during the development of excitability. However, the small Na^+ current (I_{Na}), which was present when the action potential first appeared, increased twofold in density. Ca^{2+}-dependent K^+ current (I_{Kc}) density increased twofold, and the voltage-dependent K^+ (delayed rectifier) current (I_{Kv}) density increased threefold and became activated almost twice as fast (O'Dowd et al., 1988). I_{Kv} was emerging as a major contributor to maturation of the action potential. Single channel recordings identified 15 and 30 pS conductance classes of voltage-dependent K^+ channels in both young and mature neurons. We detected no changes in open probability of these channels, enabling calculation that the number of channels had increased threefold. Only the 30 pS class showed an increase in its activation rate (Harris et al., 1988). These single channel recordings showed that the changes in I_{Kv} during maturation reflected increases in the number of channels and not increases in single channel conductance.

In an elegant study, Angie Ribera discovered the period during which transcription was required for the differentiation of I_{Kv} and for the maturation of the action potential. A nine-hour application of a reversible RNA synthesis inhibitor to cultured neurons irreversibly prevented both events. Removal of the inhibitor after nine hours allowed resumption of RNA synthesis, but I_{Kv} did not appear and the action potential remained long in duration. Instead, a different K^+ current, I_{KA}, which normally appears later than I_{Kv}, was now expressed, albeit delayed in its appearance by the nine hours of inhibited RNA synthesis. These results demonstrated a critical period for transcription essential for I_{Kv} to appear. After the critical period, no recovery of I_{Kv} was observed (Ribera & Spitzer, 1989; 1990).

The Development of Excitability: Reconstruction of the Developing Action Potential

Voltage-clamp recordings of amphibian spinal neurons during development had revealed changes in I_{Ca}, I_{Na}, I_{Kc}, I_{Kv}, and I_{KA}. To determine whether these changes were sufficient to produce the changes in action potential duration and ionic dependence that we had observed, Shawn Lockery and I constructed a HodgkinHuxley model of the electrical excitability of these neurons. During the day, Shawn was doing postdoctoral work with Terry Sejnowski, and I was doing experiments in the lab, so we worked in the evenings. Had we identified all the currents underlying the young and mature action potentials, or were additional currents required? Does I_{Kv} make the largest contribution? Could changes in other currents be even

more important? The model showed that the equations describing the voltage-clamped currents of young and mature neurons generated action potentials that were appropriate in duration and ionic dependence for each developmental stage. Moreover, the observed changes in the currents were quantitatively sufficient to produce the changes in the action potential observed during development. The effect of the change in each current was detectable in the model. Notably, the increase in amplitude of I_{Kv} had the largest effect. These results were hugely rewarding. The model showed that the currents we had characterized could account for all our observations of action potentials during development. Furthermore, changes in action potential duration could be achieved with changes in kinetics rather than in the amplitude of I_{Kv} or with changes in amplitudes of other currents (Lockery & Spitzer, 1992). How I_{Kv} came to have such an important role in development remains a subject for future study.

The Development of Excitability: K^+ Channels Underlying I_{Kv}

We began analyzing the expression of different K^+ channel genes to identify the ones that underlie I_{Kv}, which had proved to be the workhorse in shortening the action potential and altering its ionic dependence. xSha2 (xKv1.2) was the first *Xenopus* K^+ channel gene to be cloned. xSha2 was expressed in the nervous system at the neurula stage and encoded a delayed rectifier current when expressed in oocytes. However, it was a relatively rare transcript, suggesting that it would make only a small contribution to I_{Kv} (Ribera, 1990). Single-cell reverse transcription–polymerase chain reaction (RT-PCR) analysis of K^+ channel gene expression following recording of I_{Kv} during its development in culture identified transcripts of xKv1.1 and xKv2.2, which encode delayed rectifier current, but neither was expressed throughout the development of I_{Kv} (Gurantz et al., 1996). We also isolated xKv.3.1, which encoded a delayed rectifier current in oocytes and was upregulated in the nervous system throughout the period in which I_{Kv} develops *in vivo*. xKv3.1 was expressed in 40 percent of neurons in culture, suggesting that it contributes to maturation of I_{Kv} and to shortening of the action potential (Gurantz et al., 2000). To assess its contribution directly, we used antisense cRNA to xKv3.1 to suppress expression of this gene in the spinal cord. These experiments reduced the amplitude and rate of activation of I_{Kv} and increased the duration of the action potential in 40 percent of the neurons, matching the expression of xKv3.1 that we had observed in cultured neurons (Vincent et al., 2000). These findings, along with Angie Ribera's results from her lab at the University of Colorado, indicated that heterogeneous K^+ channel gene expression gives rise to homogenous I_{Kv} current. It was satisfying to have an understanding of the development of the action potential at the level of expression of single K^+ channel genes.

Intracellular Ca^{2+} Transients

We expected that the influx of Ca^{2+} during long-duration, Ca^{2+}-dependent action potentials would raise intracellular levels of Ca^{2+}. Janet Holliday joined the lab for her postdoc, and we began imaging intracellular Ca^{2+} with fura-2AM to study the effects of Ca^{2} influx more directly. We first discovered that neurons exhibit a transient elevation of Ca^{2+} in response to a single Ca^{2+}-dependent action potential. We also observed spontaneous, transient elevations of intracellular Ca^{2+} in culture during early development at the time when action potentials produce the greatest Ca^{2+} influx. The imaging studies further revealed that rapid Ca^{2+} elevations, both in the nucleus and in the cytosol, were primarily due to Ca^{2+}-induced Ca^{2+}-release from intracellular stores that was triggered by Ca^{2+} influx (Holliday & Spitzer, 1990; Holliday et al., 1991).

Xiaonan Gu wrote to me to inquire about a postdoc while he was completing his graduate work with Ken Muller. Ken recommended him with great enthusiasm and I was glad to take him on. Xiaonan was deeply insightful and extraordinarily creative. We established an exceptional partnership, working initially with cultured neurons to understand how Ca^{2+} transients are triggered, and studying the biophysical properties and function of low-voltage-activated (LVA) T-type Ca^{2+} current (I_{CaT}). There were no significant changes in threshold, peak density, kinetics, and pharmacological sensitivity of I_{CaT} during development. Spontaneous Ca^{2+} transients in young neurons assayed by fluo-3AM fluorescence were blocked by Ni^{2+} or amiloride at concentrations that specifically blocked I_{CaT}. This current had the lowest threshold of activation among other inward currents in young neurons, and mathematical simulations showed that I_{CaT} lowers the threshold of the action potential by 15 mV. We concluded that I_{CaT} can depolarize cells and trigger action potentials and that this current is part of the cascade of events leading to spontaneous elevations of intracellular Ca^{2+} in cultured neurons at early stages of differentiation (Gu & Spitzer, 1993). Michel Desarmenien, visiting on leave from the French National Centre for Scientific Research, undertook voltage clamp analysis of embryonic neurons developing *in vivo*, focusing on the Ca^{2+} and K^{+} currents that play key roles in triggering and controlling the duration of action potentials (Desarmenien et al., 1993). Reassuringly, low-voltage-activated Ca^{2+} current, I_{CaT}, I_{Kv}, and I_{KA} all developed as previously observed in culture.

Imaging *Xenopus* spinal neurons with fluo-3AM both in culture and in the intact isolated spinal cord for one hour during development identified two classes of spontaneous Ca^{2+} transients, Ca^{2+} spikes and Ca^{2+} waves, often in the same neuron. Graduate student Eric Olson joined us in these studies. We found that propagated Ca^{2+} spikes had rapid, stereotyped rise and decay, were about 10 sec in duration, depended on Ca^{2+}-dependent action potentials and occurred at a frequency of about three spikes per hour that decreased during development. Waves were located in growth cones,

had a slow nonuniform rise and decay, and were several minutes in duration. They did not depend on Ca^{2+}-dependent action potentials and occurred at frequencies of about eight waves per hour throughout development. Both spikes and waves were abolished by removal of extracellular Ca^{2+} (Gu et al., 1994). These observations raised the possibility that the two distinct signals (spikes and waves) regulate different aspects of neuronal differentiation.

Regulation of Neuronal Differentiation by Intracellular Ca^{2+} Transients

We found three distinct neuronal properties that were regulated by either Ca^{2+} waves or Ca^{2+} spikes. One property, the extent of neurite outgrowth, depended on waves. Because Ca^{2+} waves were expressed in growth cones, they were in a position to regulate the extension of neurites. We found that Ca^{2+} waves inhibited neurite outgrowth. Thus, neurites were short in the presence of extracellular Ca^{2+} when waves were present. When waves and Ca^{2+} influx were prevented, by removal of extracellular Ca^{2+}, neurites were long. Depletion of intracellular Ca^{2+} stores during development, even in the presence of extracellular Ca^{2+}, also produced long neurites. The additional effect of these manipulations on Ca^{2+} spikes proved unrelated to the growth of neurites, because selectively blocking Ca^{2+} spikes did not affect growth (Bixby & Spitzer, 1984b; Holliday & Spitzer, 1990; Holliday et al., 1991; Gu et al., 1994). The inhibiting action of Ca^{2+} waves on neurite extension was mediated by calcineurin, a Ca^{2+}-dependent phosphatase. Inhibition of this enzyme blocked the Ca^{2+}-dependent reduction in rate of outgrowth, even though the frequency of waves was not affected. Moreover, constitutive expression of *Xenopus* calcineurin led to the generation of short neurites in the absence of Ca^{2+} waves. We were able to show that calcineurin suppressed the phosphorylation of GAP-43, thereby destabilizing actin filaments and slowing neurite extension (Lautermilch & Spitzer, 2000). It was gratifying to understand the molecular basis of Ca^{2+} wave action.

The second property, the rate of activation of I_{Kv} during development, proved to depend on Ca^{2+} spikes. Blocking voltage-dependent Ca^{2+} channels or intracellular Ca^{2+}-induced Ca^{2+}-release prevented the increase in activation rate of I_{Kv}. Significantly, depletion of protein kinase C in the presence of extracellular Ca^{2+} also prevented the increase, thereby identifying a molecular mechanism for the action of intracellular Ca^{2+}. Stimulating the kinase in the absence of extracellular Ca^{2+} permitted an increase in the activation rate. Thus, Ca^{2+} spikes at early stages of development regulate the differentiation of the kinetics of I_{Kv}, perhaps by increased expression of the 30 pS channel described earlier, and modulate the dependence of action potentials on this current (Desarmenien & Spitzer, 1991).

The third property, neurotransmitter identity, also turned out to be regulated by Ca^{2+} spikes. We knew that the early differentiation of excitability in *Xenopus* spinal neurons in culture parallels their development *in vivo*.

This encouraged us to examine the maturation of gamma-aminobutyric acid (GABA) immunoreactivity in cultured neurons, using an excellent antibody that recognized GABA. We found that GABA immunoreactivity in culture developed with the same time course previously described *in vivo*, and its appearance was blocked by transcriptional inhibitors. After prolonged absence of Ca^{2+}-dependent action potentials, or prolonged depletion of Ca^{2+} stores, the number of GABAergic neurons was dramatically reduced. Importantly, this reduction in neuronal number occurred in the absence of cell death. The loss of GABA depended on sustained absence of Ca^{2+} influx. Janet Holliday and I, along with others, published a modest report of this finding, which would shape the direction of my scientific work from that point forward (Spitzer et al., 1988). Full accounts of these findings appeared subsequently (Holliday et al., 1991; Spitzer et al., 1993). Although it never occurred to us at the time, the reduction in number of GABAergic neurons would later prove to be associated with an equivalent increase in the number of neurons expressing a different transmitter, glutamate (Borodinsky et al., 2004). This occurred in the absence of neurogenesis. Looking back, I see that this finding of a reduced number of GABAergic neurons, when Ca^{2+} elevations were blocked, was the first hint of our subsequent discovery of neurotransmitter switching, which occurs in response to sustained changes in electrical activity.

The control of neuronal differentiation by Ca^{2+} spikes and waves suggested the principle of a developmental handshake involving reciprocal control, in which differentiation regulates ion flux and ion flux regulates differentiation (Spitzer, 1991). Later, Yehezkel Ben-Ari and I realized that there is a more general version of this principle. The development of the central nervous system requires intermediate stages of differentiation, providing functions that influence subsequent gene expression. We proposed that embryonic neuronal functions constitute a series of checkpoint signatures. Neurons failing to express these functions are delayed or developmentally arrested (Ben-Ari & Spitzer, 2010).

The difference in frequency of Ca^{2+} spikes and Ca^{2+} waves reminded me of the frequency coding of information in the peripheral nervous system (Adrian & Zotterman, 1926, *Journal of Physiology,* 61, 151–171). This led me to wonder whether the frequencies of spikes and waves were important for regulating expression of the three neuronal properties we were studying. Xiaonan Gu perfused cultures of *Xenopus* spinal neurons with Ca^{2+}-free medium to eliminate spontaneous Ca^{2+} transients and used computer-controlled solenoid valves to deliver pulses of Ca^{2+} medium containing K^+ to depolarize neurons. This procedure generated transient elevations of intracellular Ca^{2+} that mimicked spikes or waves, and these could be produced at different frequencies. Imposing different frequencies of Ca^{2+} elevation in this way demonstrated that the spontaneous wave frequency in the cultures was sufficient to regulate normal neurite extension, and the spontaneous spike frequency in the cultures was sufficient to produce normal

neurotransmitter expression and current maturation. Chelation of intracellular Ca^{2+}, while imposing the spontaneous frequencies of Ca^{2+} transients, demonstrated that these frequencies were also necessary for normal differentiation. These tests with different frequencies showed that the frequency of Ca^{2+} transients encoded information, like the frequency of action potentials, although the Ca^{2+} transients were 10^4 times longer in duration and less frequent than action potentials (Gu & Spitzer, 1995).

Activity-Dependent Growth Cone Motility

To what extent were our findings about the role of growth cone Ca^{2+} waves in regulating neurite outgrowth in culture relevant to axon extension in the intact spinal cord? Lateral dissections of *Xenopus* embryos allowed us to observe migrating growth cones *in vivo*. By imaging fluo-3AM loaded neurons, postdoc Tim Gomez recorded transient elevations of $[Ca^{2+}]_I$ with the same properties of growth cone waves we had seen *in vitro*. We found that growth cones of sensory Rohon-Beard neurons, motor neurons, and interneurons exhibited different migration velocities at different points in their trajectories in the spinal cord. For each class of neurons, the frequency of their growth cone Ca^{2+} transients was inversely proportional to the velocity of growth cone migration. Photoactivation of an intracellular Ca^{2+} chelator in slowly migrating growth cones suppressed Ca^{2+} transients and increased the rate of outgrowth. Photoactivation of intracellular caged Ca^{2+} in rapidly migrating growth cones generated Ca^{2+} transients and decreased the rate of outgrowth. At turning points, growth cones increased the frequency of Ca^{2+} transients and paused, as if sampling the environment for critical cues. We concluded that these growth cone Ca^{2+} transients provide a natural signaling mechanism that regulates axon extension and perhaps axon guidance (Gomez & Spitzer, 1999). Imaging growth cone Ca^{2+} transients in primary motor neurons of transparent zebrafish embryos with GCaMP3 also revealed differences in frequency that were correlated with the location that the growth cones had reached. High-frequency bursts of Ca^{2+} transients were associated with growth cone stalling at turning points. Suppression of Ca^{2+} transients by Kir2.1 K^+ channel expression in single zebrafish primary motor neurons caused the neurons to make pathfinding mistakes (Plazas et al., 2013). These results raised the question of what cues were eliciting the Ca^{2+} transients.

The Origin of Spontaneous Ca^{2+} Transients in Growth Cones

Growth cone filopodia are thin sensory-motor protrusions from the palm of the growth cone that detect environmental cues and translate them into changes in motility. High-speed (8 Hz) confocal imaging of magnified regions of growth cones of cultured *Xenopus* spinal neurons loaded with fluo-4AM revealed brief

(0.3-second duration) Ca^{2+} transients in filopodia. Their frequency, but not their amplitude or duration, depended on the identity and concentration of the environmentally relevant proteins on which the neurons were cultured. These filopodial transients occurred at a frequency of one to nine per minute in active filopodia and were rapidly propagated to the palm of the growth cone. The lifetimes of filopodia were proportional to the frequency of their filopodial transients. Higher frequencies initiated the larger Ca^{2+} transients in the palm of the growth cone that we previously had shown to suppress axon extension. Variations in the frequency of filopodial transients across the filopodia of a growth cone, when it encountered a boundary between two different substrate proteins, caused the growth cone to turn away from the side with the higher frequency. Thus, the filopodial Ca^{2+} transients, by way of a frequency code, were poised to regulate both growth cone extension and the dependence of turning on environmental cues (Gomez et al., 2001).

We also found that Ca^{2+} transients and transient elevations of cAMP were interdependent in the cell bodies of cultured *Xenopus* spinal neurons (Gorbunova & Spitzer, 2002). This discovery prompted investigation of the interactions between these two second messengers in the filopodia of the growth cones of these neurons. Postdoc Xavier Nicol found that Netrin-1, which attracts growth cones to the midline of the nervous system, drives a transient elevation of cAMP in filopodia, which does not depend on Ca^{2+}, and briefly increases the frequency of filopodial Ca^{2+} transients. In contrast, Netrin-1 in the palm of the growth cone drives a transient Ca^{2+}-dependent increase in cAMP and a sustained increase in the frequency of the Ca^{2+} transients that inhibit axon extension. Optogenetically generated cAMP transients in filopodia, but not in the palm of the growth cone, caused growth cone turning, and the angle of the turn was proportional to the frequency of imposed filopodial cAMP transients. We confirmed *in vivo* the relevance of these *in vitro* observations by spatially restricting the optogenetic stimulation of cAMP transients to filopodia. This was sufficient to steer growth cones to the midline when the Netrin-1 receptor was blocked (Nicol et al., 2011). It was clear that electrical activity, in different forms, regulates many aspects of development of the nervous system (Spitzer, 2006). Further investigation of activity-dependent growth cone motility was inviting, as it harked back to my enthusiasm for working on the wiring problem. However, our study of activity-dependent changes in neurotransmitter expression was heating up and began to consume my attention.

The Discovery of Activity-Dependent Transmitter Switching During Development

Laura Borodinsky joined the lab as a postdoctoral fellow, and we discovered transmitter switching in the developing spinal cord. Laura had the impressive ability to find the right way to do the right experiments.

Previous studies had demonstrated that neurotransmitter identity is specified by intrinsic transcription factors, as Tom Jessell and his colleagues had discovered (Tanabe et al., 1998, *Cell*, 95, 67–80), and modified by cytokines, elevation of intracellular Ca^{2+}, and neurotrophic factors, as Paul Patterson, Story Landis, and their collaborators had shown (Patterson & Chun, 1974, *Proceedings of the National Academy of Sciences USA*, 71, 3607–3610; Walicke & Patterson, 1981, *Journal of Neuroscience*, 1, 343–350; Landis and Keefe, 1983, *Developmental Biology*, 98, 349–372). Ed Furshpan and Dave Potter provided functional evidence for a change in transmitter within single sympathetic neurons (Furshpan et al., 1976, *Proceedings of the National Academy of Sciences USA*, 73, 4225–4229; Potter et al., 1986, *Journal of Neuroscience*, 6, 1080–1098). When we imaged fluo-4AM-loaded glutamatergic Rohon-Beard neurons, glycinergic dorsolateral and GABAergic ventral interneurons, and cholinergic ventral motoneurons in the embryonic *Xenopus* neural tube, we found that each class of neurons exhibited a specific pattern of spontaneous Ca^{2+} spike activity. Sustained suppression or enhancement of this activity, either by misexpression of K^+ or Na^+ channels or with pharmacological antagonists or agonists, altered the number of neurons expressing each of the four neurotransmitters, as identified by immunocytochemistry. Suppressing Ca^{2+} spike activity increased the number of neurons expressing glutamate and acetylcholine, the canonical excitatory transmitters, and decreased the number of neurons expressing GABA and glycine, the typical inhibitory transmitters. In contrast, enhancing Ca^{2+} spike activity had the opposite effect. It decreased the number of neurons expressing the excitatory transmitters and increased the number of neurons expressing the inhibitory transmitters (Borodinsky et al., 2004).

These findings suggested that activity-dependent regulation of neurotransmitter identity is homeostatic. Suppression of electrical activity appeared to be compensated by an increase in the number of excitatory neurons and a decrease in the number of inhibitory neurons. In a similar manner, enhancement of activity was compensated by an increase in the number of inhibitory neurons and a decrease in the number of excitatory neurons. Significantly, the total number of Rohon-Beard sensory neurons and motoneurons, for which independent markers of neuronal identity were available, was unchanged even while the number of neurons expressing glutamate and acetylcholine was altered (markers for the interneurons were not known at that time).

These results compelled us to conclude that neurons, responding to changes in activity, were changing their transmitters. Similar changes in transmitter identity were observed in culture, where we could show that the transmitter changes were functional. When we recorded spontaneous synaptic currents, we found that the proportion of neurons generating glutamatergic or cholinergic currents was indistinguishable from the proportion of neurons expressing glutamatergic or cholinergic immunoreactivity.

Roles for neurogenesis or apoptosis seemed unlikely to be relevant because the number of Rohon-Beard neurons and motor neurons did not change following changes in Ca^{2+} spike activity. It was difficult to avoid the conclusion that neurotransmitter identity can be plastic *in vivo*, at least in the embryonic nervous system.

The Discovery of Transmitter-Receptor Matching

The discovery of transmitter switching created a quandary with respect to the postsynaptic transmitter receptors. A mismatch between the new transmitter and the old receptors would render the synapse inoperative. Fortunately, Laura Borodinsky's examination of embryonic *Xenopus* striated muscle cells revealed that before innervation they normally expressed receptors for glutamate, GABA, and glycine in addition to acetylcholine. During normal differentiation and innervation of the muscle cells, acetylcholine receptor expression was sustained and the other receptors were downregulated. When the motoneuron transmitter was changed to glutamate, GABA, or glycine by altering Ca^{2+} spike activity, the appropriate cognate noncholinergic receptors were upregulated. In these conditions, glutamatergic, GABAergic, and glycinergic synaptic currents as well as nicotinic cholinergic currents could all be recorded from the muscle cells. When neurons were cocultured with muscle cells in the presence of extracellular Ca^{2+} (but not in its absence), which transmitter receptors were upregulated depended on which neurotransmitter was expressed by the neurons (Borodinsky & Spitzer, 2007). Thus, prior to innervation and in the absence of synapses, the selection of receptors seemed to depend on diffusible factors from the neurons.

We later tested the hypothesis that the diffusible factors include the neurotransmitters themselves. Using a functional assay, we found that if embryonic vertebrate skeletal muscle cells are cultured before they are innervated, then sustained application of glutamate to the cells is necessary and sufficient to upregulate ionotropic glutamate receptors from the pool of receptors that are normally expressed at low levels. Extending this result *in vivo*, by using implanted agarose beads impregnated with 2 mM glutamate to achieve sustained local diffusion of agonist, we saw that application of glutamate to embryonic skeletal myotomes was necessary and sufficient for upregulation of the GluN1 NMDA receptor subunit. These results suggested a mechanism by which neuronal release of a new (switched) transmitter upregulates the postsynaptic expression of appropriate, matching transmitter receptors (Hammond-Weinberger et al., 2020).

The Origin of Spontaneous Ca^{2+} Spike Activity

We now wanted to understand the cause of the spontaneous Ca^{2+} spike activity that, when suppressed or enhanced, leads to transmitter switching

during development. It seemed possible that neurotransmitters could themselves regulate production of Ca^{2+} spikes. Cory Root, my creative and productive lab manager, took up this challenge (he would later return to UC San Diego as a faculty member). We found that, at the neural tube stage of development, both Rohon-Beard neurons and motoneurons coexpress glutamate and GABA. At the later tailbud stage, Rohon-Beard neurons selectively express glutamate and motoneurons selectively express acetylcholine, while interneurons selectively express GABA. Neither neurogenesis nor cell death could account for these changes. At the neural tube stage, both glutamate and GABA stimulated the generation of Ca^{2+} spikes via metabotropic receptors and activation of protein kinases A and C. Preventing the expression of GABA, or blocking GABA and glutamate receptors, lowered the frequency of Ca^{2+} spikes. This had the effect of increasing the number of neurons that express the excitatory transmitters, acetylcholine and glutamate, and of decreasing the number of neurons that express the inhibitory transmitters, GABA and glycine (Root et al., 2008). These findings answered our persisting question about the origin of spontaneous Ca^{2+} spike activity early in development. Neurotransmitters expressed early in development cause this spontaneous activity.

Environmental Stimuli Cause Transmitter Switching That Changes Behavior

At this point we understood that experimental manipulations of Ca^{2+} spike activity can regulate which neurotransmitter is expressed. But we had no idea whether transmitter expression in a neuronal population can also be specified by natural environmental stimuli. Furthermore, we did not know if transmitter switching had relevance for behavior. Davide Dulcis, who had a marvelous talent for doing ingenious and revealing experiments, joined the lab as a postdoc at this point. To address these questions, we first studied neurons in the ventral suprachiasmatic nucleus that in teleosts, amphibia, and primates regulates neuroendocrine pituitary function in response to light. In *Xenopus* larvae, we found that increasing or decreasing exposure to light for as little as 10 minutes regulated camouflage by clustering or dispersing the distribution of melanin in melanocytes. Skin pigmentation decreased in the light and increased in the dark. The effect of light on pigmentation was prevented by blocking D2 dopamine receptors.

Neurons in the ventral suprachiasmatic nucleus of the larvae normally exhibit Ca^{2+} spikes similar to those observed in the neural tube. We found that light, by increasing Ca^{2+} spike activity, caused the appearance of dopamine specifically in neurons that already expressed neuropeptide Y (NPY). Intriguingly, these newly appearing dopaminergic neurons already projected to the melanotrope cells that regulate melanocytes. After dopamine was expressed as a cotransmitter, the melanotrope cells expressed

D2 dopamine receptors. NPY expression continued. We did not investigate whether another cotransmitter disappeared when dopamine appeared.

When the endogenous population of dopaminergic neurons was ablated, there was no change in skin pigmentation in response to a brief period of illumination. However, when these larvae were exposed to bright illumination for two hours, the newly appearing dopaminergic neurons rescued the camouflage regulation. This result demonstrated that the new dopaminergic neurons have relevance for behavior. We concluded that a natural stimulus, light, can alter the number of amine-transmitter-expressing neurons in the brain at larval stages of development and control a natural behavior, camouflage (Dulcis & Spitzer, 2008).

We later discovered another instance in which environmental stimuli drive transmitter switching and change behavior, when we studied changes in social preference of *Xenopus* larvae that ordinarily result from sustained exposure to kinship odorants. After normal development in the presence of siblings, sibling-raised larvae cluster in the water by swimming either toward a briefly available (10 minutes) kin odorant (sibling-conditioned water) or away from a non-kin odorant (nonsibling conditioned water). However, after sustained exposure (24 hours) to water containing kinship-signaling odorants released from nonsiblings, sibling larvae change their social preference and cluster with nonsiblings.

We found that this altered behavior was mediated by changes in the expression of dopamine and GABA in granule and periglomerular interneurons of the accessory olfactory bulb. In both granule and periglomerular cells, sustained exposure to kin odorants increased the number of interneurons expressing dopamine and decreased the number of interneurons expressing GABA. GABAergic neurons expressing the Pax6 transcription factor became dopaminergic neurons expressing Pax6 and no longer expressed GABA. In contrast, sustained exposure to non-kin odorants decreased the number of neurons expressing dopamine and increased the number of neurons expressing GABA. Blocking Na^+-dependent action potentials or suppressing elevations of intracellular Ca^{2+} prevented these changes in transmitter expression.

We then looked for a causal connection between the newly specified transmitter and changes in kinship recognition. We first ablated endogenous dopaminergic neurons in the accessory olfactory bulb of sibling-raised larvae. This treatment prevented attraction to kin odorants, suggesting that dopaminergic neurons in the bulb are ordinarily necessary for normal processing of the kinship odorants that elicit attraction to siblings. We next exposed these larvae to kin odorants for 24 hours to induce the expression of dopamine in neurons that initially expressed GABA. This treatment restored the attraction to kin odorants. Rescue of sibling preference suggested that the newly specified dopamine-expressing neurons were sufficient to support kinship recognition (Dulcis et al., 2017).

Reserve Pool Neurons

I was fascinated by the finding that transmitter switches were associated with changes in behavior. Later work would continue to demonstrate that the switches caused the changes in behavior, as we were able to show that overriding the switches prevented the behavioral changes. However, transmitter switching during development occurred only in a subset of the neurons in a nucleus or region. Davide Dulcis and I proposed that these cells be designated reserve pool neurons. Reserve pool neurons already participate in neuronal circuits with afferent and efferent connections. In response to sustained activity, they acquire or lose or switch neurotransmitters, thus endowing circuits with a broader range of function. It was as if they had a day job and potentially a second night job and that activity could recruit them for this second job. In the case of a transmitter switch, reserve pool neurons would then give up their day job. Reserve pool neurons may be endowed with special signal transduction pathways not possessed by their nonswitching neighbors (Dulcis & Spitzer, 2011). Alternatively, their synaptic connections may enable them to generate higher or lower levels of activity than their neighbors, and in that case, they may not have special signal transduction machinery. I hope to understand what gives reserve pool neurons their special status.

The Discovery of Activity-Dependent Transmitter Switching in the Adult

Up to this point, I had supposed that transmitter switching was solely a developmental form of synaptic plasticity. However, when we searched for it in the adult rat brain, we were in for a big surprise. We knew that, during development, neurons in the suprachiasmatic nucleus can change their transmitter in response to light (Dulcis & Spitzer, 2008). Working from this finding, we tested the effect of a one-week exposure to long-day, balanced-day, or short-day photoperiods on transmitter expression and behavior. Rats are nocturnal and prefer short-day (long-night) photoperiods. We found that the paraventricular nucleus and several other hypothalamic nuclei increased the number of dopaminergic neurons during short-day photoperiods and decreased the number of dopaminergic neurons during long-day photoperiods, when compared to the number resulting from balanced-day photoperiods. The number of dopaminergic neurons resulting from long- and short-day photoperiods was matched by corresponding increases and decreases in the number of neurons expressing somatostatin. Thus, after exposure to the three different photoperiods, the number of somatostatin neurons was inversely related to the number of dopaminergic neurons. There was no change in adult neurogenesis or apoptosis in any of these conditions. Individual neurons appeared to be reciprocally switching transmitter expression between dopamine and somatostatin.

Somatostatin neurons of the paraventricular nucleus, as well as dopaminergic neurons, make synapses on corticotropin-releasing factor (CRF) neurons distributed along the third ventricle. After the stressful long-day photoperiod, and along with the decrease in number of dopaminergic neurons, dopamine D2 receptors on these CRF neurons were downregulated. In contrast, after the short-day photoperiod, and in parallel with an increase in number of dopaminergic neurons, the D2 receptors were upregulated. To our surprise, the level of somatostatin receptors did not change. After the short-day photoperiod, the level of CRF in the cerebrospinal fluid was lower, presumably because its release could be inhibited by both dopamine D2 receptors and somatostatin receptors. As expected, the level of CRF was higher after the long-day photoperiod, when D2 receptors were rare, and release could be inhibited only by somatostatin receptors. These findings reassured us that the D2 dopamine-somatostatin transmitter switch was functional.

To investigate the behavioral effects of the exposure to different photoperiods, we tested rats on the elevated plus maze and in the forced swim test, widely used assays of stress. Relative to controls exposed to the balanced-day photoperiod, rats with short-day exposure exhibited less stress. They spent more time exploring the open arm of the maze and spent a longer time swimming before becoming immobile. Long-day exposure produced the opposite effects. To determine whether these behavioral changes were specifically related to changes in the number of dopaminergic neurons, we ablated these neurons in animals maintained on the balanced-day photoperiod. This procedure increased stressful behavior in the same way as exposure to long-day photoperiods. Rats spent less time exploring the open arm of the elevated plus maze and less time swimming before becoming immobile. These findings showed that reducing the number of dopaminergic neurons was sufficient to lead to stress-like behavior.

We also asked whether photoperiod-dependent, hypothalamic transmitter switching was necessary for the observed changes in behavior. We first ablated dopaminergic neurons in the hypothalamic nuclei and then exposed animals to the short-day photoperiod to recruit more neurons to become dopaminergic. The stress-like behavior that we had seen after ablation of dopaminergic neurons was now reversed. This behavioral rescue was prevented by acute, local infusion of dopamine receptor antagonists, showing that the rescue depended on the newly recruited dopaminergic neurons and not on some other mechanism (Dulcis et al., 2013). These findings made a big impression on me, suggesting a relationship to the characteristics of seasonal affective disorder, a form of clinical depression that is more common at high latitudes in association with the short-photoperiod days of winter.

We followed up this notion in a long-range collaboration with colleagues in Melbourne after discussion of the relevance of our findings to humans.

Thinking of high latitudes where photoperiod durations undergo wide swings over the course of a year, we secured postmortem brains from residents of Scotland, which we obtained from the Edinburgh University Brain Bank. These individuals had died from heart disease or cancer during either the summer (long-day photoperiod) or during the winter (short-day photoperiod). We found that the density of neurons expressing dopamine and its transporter was higher in the midbrains of those who had died in the summer than in the midbrains of those who had died in the winter. This study established an association in humans between photoperiod and the number of midbrain dopaminergic neurons and suggested to us that dopaminergic neurotransmitter switching might underlie this association (Aumann et al., 2016).

Beneficial Effects of Switching

Our discovery of transmitter switching in the adult mammalian brain was astounding to me, because neuronal transmitters were considered to be fixed and unchanging in the mature nervous system. I thought that transmitter switching might have explanatory power for a whole range of instances in which there is loss or gain of function in response to experience. Evidently, transmitter switching was not a process restricted to the developing nervous system. Where else in the brain could it occur and in response to what kinds of experience? Thinking about the path forward stimulated consideration of the model system that could best address the next set of questions. We quickly turned to adult mice, recognizing the availability of genetic tools with which to pursue our studies. I wondered whether the behavioral effects of transmitter switching resulted only from aversive experience, or whether rewarding experience could induce transmitter switches as well and, if so, was the resulting behavior beneficial? Hui-quan Li came to the lab as a post-doc, and we tackled this question.

Mice love to run. When running wheels are placed outdoors, infrared video cameras have revealed that the wheels are frequently used by wild mice at night. Moreover, running wheel use in the wild matches running wheel use by laboratory mice. We found that one week of voluntary wheel running in the lab induced transmitter switching from acetylcholine to GABA in neurons of the caudal pedunculopontine nucleus, which is important for gait and balance. The switch involved a decrease in the number of neurons expressing acetylcholine and a matching increase in the number of neurons expressing GABA. We next genetically marked cholinergic neurons to identify ones that had switched their transmitter. This method permanently labeled cholinergic neurons with mRuby2 fluorescent protein, even those that lost acetylcholine as a result of running. We could then score the number of mRuby2-labeled fluorescent neurons that expressed GABA after running. The results showed unambiguously that formerly cholinergic

neurons now expressed GABA. No neurogenesis or apoptosis was detected. The switch was prevented by expressing K^+ channels in the cholinergic neurons to suppress the generation of action potentials, demonstrating that the switch was activity-dependent.

Following sustained running, mice also exhibited enhanced learning of motor skills, mastering the accelerating rotarod more rapidly and accommodating to the narrow balance beam more quickly than mice without access to a running wheel. Interestingly, we found that switching neurons project to the substantia nigra, ventral tegmental area, and the thalamus, all areas that regulate motor skill learning. Enhanced performance of the motor skills was sustained for at least two weeks, although the transmitter switch spontaneously reversed at the end of a week of rest following the one week of running. Because the behavior persisted even when the switch had reversed, this finding suggested that plasticity had occurred downstream of the switching neurons, perhaps at one of the targets of the switching neurons, such as the substantia nigra.

To determine whether transmitter switching was necessary for the beneficial effect of running on motor skill learning, we sought to override the switch in transmitter identity. First, prior to access to the running wheel, we introduced the gene encoding choline acetyltransferase into genetically marked cholinergic neurons to sustain expression of acetylcholine in neurons that otherwise would lose it after running. Now, one week of running no longer enhanced motor skill learning. In parallel experiments, we introduced a gene encoding an inhibitor of the gene encoding glutamic acid decarboxylase to prevent the appearance of GABA after running. Again, we found that one week of running no longer enhanced motor skill learning. These experiments showed that either compensating for the loss of acetylcholine, or preventing the gain of GABA, blocked the beneficial effect of sustained running. Clearly, rewarding experience stimulated transmitter switching, and the effects were beneficial (Li & Spitzer, 2020). We began to suspect that transmitter switching could be induced by many kinds of experience, particularly when they were sustained in duration.

Mechanisms of Transmitter Switching in Development: Transcription Factor Activation

I wanted to understand the cellular and molecular mechanisms of transmitter switching, as this information might make it possible to regulate transmitter switching for beneficial purposes. The preponderance of glutamate and GABA as neurotransmitters recommended study of the glutamate-to-GABA switch that we had discovered in the embryonic *Xenopus* spinal cord. Because Ca^{2+} spike activity initiates transmitter switching, when Alicia Güemez-Gamboa joined the lab as a postdoc, we began by investigating the mechanism by which Ca^{2+} spike activity is transduced. Graduate student

Lin Xu first suppressed Ca^{2+} spikes in single neurons to determine whether glutamate-to-GABA switching is cell autonomous. Transmitter switching did not occur, suggesting that it is regulated by the level of Ca^{2+} spike activity in surrounding neurons. We then found that brain-derived neurotrophic factor (BDNF) was expressed in the spinal cord and that increasing Ca^{2+} spike activity in cultures of spinal neurons caused the release of BDNF. Activation of TrkB receptors by BDNF in the spinal cord triggered a mitogen-activated protein kinase signaling cascade, which concluded in cJun N-terminal kinase-mediated activation of the cJun transcription factor and glutamate-to-GABA switching. Introduction of BDNF, after suppression of Ca^{2+} spikes, drove the switch, increasing the number of GABAergic neurons and decreasing the number of glutamatergic neurons. Inhibiting production of BDNF or blocking activation of TrkB receptors, reversed the switch, increasing the number of glutamatergic neurons and decreasing the number of GABAergic neurons. These findings identified the initial transduction mechanism for activity-dependent transmitter switching in developing spinal neurons (Güemez-Gamboa et al., 2014).

Postdoc Kurt Marek and graduate student Lisa Kurtz led the search for gene expression regulated by this signaling cascade. Using a functional screen, we identified *tlx3* (the *t-cell leukemia homeobox-3* gene). We found that *tlx3* expression depended on Ca^{2+} spike activity. Additionally, *tlx3* was expressed in Rohon-Beard neurons, which we had shown to undergo the glutamate-to-GABA switch. Thus, this gene was expressed in the right place at the right time to regulate the switch. Knocking down *tlx3* function with morpholinos produced the switch, increasing the number of GABAergic neurons in the spinal cord and decreasing the number of glutamatergic neurons. Overexpression of *tlx3* had the opposite effect on the transmitter switch, increasing the number of glutamatergic neurons and decreasing the number of GABAergic neurons. These results showed that *tlx3* can function as a switch that specifies the level of expression of glutamatergic and GABAergic phenotypes.

To learn how *tlx3* was regulated by activity, we surveyed the promoter region of the frog, mouse, and human *tlx3* genes for conserved transcription factor binding sites. This led us to identify a variant form of the cAMP response element (CRE), with high conservation, suggesting that this nucleotide sequence could be important for the regulation of *tlx3* transcription. We next directly assessed the role of cJun in transmitter switching by overexpressing wild-type or dominant-negative cJun. Overexpressed cJun produced the switching that we had observed following increased Ca^{2+} spike activity, increasing the number of GABAergic neurons and decreasing the number of glutamatergic neurons, similar to the results after *tlx3* knockdown. In contrast, overexpressed dominant-negative cJun caused switching in the opposite direction, decreasing the number of GABAergic neurons and increasing the number of glutamatergic neurons, similar to the results

after *tlx3* overexpression. We found further that Ca^{2+} spike activity signals through cJun phosphorylation and that phosphorylated cJun binds to the CRE site in the *tlx3* promoter. The results supported a model in which Ca^{2+} spike activity culminates in regulation of the level of cJun phosphorylation, and phosphorylated cJun binding to the CRE site modulates transcription of *tlx3* to achieve the transmitter switch. These experiments suggested a framework for understanding the mechanism of transmitter switching (Marek et al., 2010). Activity-dependent binding of a transcription factor regulates the transcription of a gene, *tlx3*, which controls the number of neurons that express glutamate vs. GABA.

Postdoc Michaël Demarque extended our understanding of the molecular mechanism of activity-dependent transmitter switching and related it to changes in behavior. We investigated in *Xenopus* larvae the conditions under which the number of serotonergic neurons changes. We found that presumptive serotonergic neurons in the developing dorsal raphe, which express the xLmx1b transcription factor but not tryptophan hydroxylase (the serotonin synthetic enzyme), generated spontaneous Ca^2 spike activity. Suppressing Ca^{2+} spike activity increased the number of neurons expressing the xLmx1b transcription factor, as well as tryptophan hydroxylase, whereas the number of neurons expressing the upstream Nkx2.2 transcription factor did not change. Increasing Ca^{2+} spike activity decreased the number of neurons expressing xLmx1b and tryptophan hydroxylase, again without effect on expression of Nkx2.2. Neuronal proliferation was not affected. Directly overexpressing xLmx1b also increased the number of serotonergic neurons, while suppressing xLmx1b expression reduced their number. Ca^{2+} spike activity appeared to exert its effects on the number of serotonergic neurons through the expression of xLmx1b (Demarque & Spitzer, 2010).

We now turned to the study of fictive swimming, because of the well-described role of serotonin in its modulation. Fictive swimming patterns consist of periods of successive action potential bursts in ventral root recordings, called swimming episodes, which are followed by periods of inactivity. Bath application of serotonin reversibly decreased the duration of swimming episodes. Suppressing Ca^{2+} spike activity to increase the number of serotonergic neurons produced a similar decrease in swimming episode duration. We knocked down the xLmx1b transcription factor to determine if its expression was responsible for the effects of Ca^{2+} spike activity on both the number of serotonergic neurons and the duration of swimming episodes. Now the number of neurons expressing xLmx1b and tryptophan hydroxylase was reduced and swimming episodes were longer in duration. These results indicated that the appearance of more serotonergic neurons can influence neuronal activity (bursts of action potentials in ventral roots) at the network level. In the intact larvae, free-swimming episodes varied in duration as predicted from analysis of fictive swimming. Overexpressing xLmx1b decreased the duration and the distance of free swimming, and

knocking down xLmx1b increased both duration and distance. Although we did not search for a switch partner that disappears with the appearance of serotonin, these results showed that, in *Xenopus* larvae, Ca^{2+} spikes regulate xLmx1b expression, which specifies the number of serotonergic neurons, and the serotonergic neurons alter swimming behavior (Demarque & Spitzer, 2010).

Mechanisms of Transmitter Switching in Development: microRNA Expression

Later, as we thought more about potential mechanisms regulating transmitter switching, I wondered whether coordinated regulation of the loss of one transmitter, and the gain of another, might involve the action of microRNAs (miRs). Giordano Lippi, then a postdoc with Darwin Berg, had helped us appreciate that miRs can function to suppress expression of one gene and silence the repressor of another, thus activating the second gene, which is the scenario in a transmitter switch. Accordingly, as we progressed in studying the social preference of *Xenopus* larvae to kin and non-kin siblings, described earlier, we profiled small RNA molecules to identify miRs in the accessory olfactory bulb that were differentially regulated by kinship exposure. We first validated the top candidates by qPCR. We then narrowed the analysis to identify those candidates that directly target the *Pax6* and *Bcl11b* genes, which are determinants of the dopaminergic and GABAergic phenotypes. We found that increased miR-375 and miR-200b levels in nonsibling conditions could contribute both to the reduction of dopaminergic neurons by suppressing expression of the stimulatory Pax6 transcription factor and to the increase in GABAergic neurons by suppressing production of the inhibitory Bcl11b transcription factor. Application of antagonists to miR-375 and miR-200b showed that changes in the levels of the transcripts of each miR were sufficient to drive changes in the number of dopaminergic or GABAergic neurons *in vivo* (Dulcis et al., 2017). Although the signaling cascade leading from Ca^{2+} spike activity to miRNA expression remains to be worked out, the results raised the possibility that microRNAs have a significant role in transmitter switching.

Mechanisms of Transmitter Switching in the Adult

Up to this point, what we knew about the mechanism of neurotransmitter switching came from our work on *Xenopus*. To study the mechanism in the case of the adult mammalian brain, we began by analyzing the activity-dependence of the dopamine-to-somatostatin switch induced by long-day photoperiod stress. Graduate student Da Meng found that suppressing activity of dopaminergic neurons in the paraventricular nucleus of the hypothalamus (PaVN) in the adult rat prevented the loss of dopamine expression we had previously observed (Dulcis et al., 2013). Interestingly, the neurons that

lost dopamine in response to the long-day photoperiod were drawn from the 50 percent of the dopaminergic neurons that normally coexpress glutamate. We noted that the CRF neurons innervated by dopaminergic neurons of the PaVN express both inhibitory dopamine D2 receptors and excitatory glutamate receptors. Accordingly, loss of D2 receptors following exposure to the long-day photoperiod could account for the increased release of CRF and the ensuing stress behaviors we had reported. We also found that suppressing activity of all PaVN excitatory glutamatergic neurons decreased the number of inhibitory PaVN dopaminergic neurons. The match of decreased excitation with decreased potential for inhibition indicated homeostatic regulation of transmitter expression in the PaVN, similar to the homeostatic regulation we had seen in the spinal cord of *Xenopus* larvae (Borodinsky et al., 2004). Thus, electrical activity regulates transmitter switching and homeostasis in the adult brain, as it does in the developing nervous system (Meng et al., 2018; Li & Spitzer, 2020). Additional studies will reveal the extent to which other aspects of regulation in the adult are the same as those identified for transmitter switches during development.

Current Research

Several ongoing projects in the lab are investigating the role of transmitter switching in mouse models of clinical disorders. One study addresses the mechanism underlying the generalized fear that results from footshock stress, which has been used as a model of post-traumatic stress disorder (PTSD). Following footshock, serotonergic neurons in the lateral wings of the dorsal raphe switch their cotransmitter from glutamate to GABA, as we also observe in postmortem human brain tissue of individuals with PTSD. The loss of glutamate and gain of GABA occur in the same neurons, and this transmitter switch appears to cause generalized fear. Expressing exogenous glutamate partially reduces generalized fear, and suppressing expression of GABA completely erases it. Prompt administration of fluoxetine directly after the footshock, but not later, blocks both the transmitter switch and acquisition of generalized fear (Li et al., 2021).

Another study investigates whether neurotransmitter switching in mice, driven by drugs of abuse, can account for changes in cognitive function. Repeated administration of phencyclidine or methamphetamine causes pyramidal neurons in the prelimbic cortex to express GABA and lose expression of glutamate, indicating that a neurotransmitter switch has occurred. Preventing these neurons from gaining GABA prevents the appearance of drug-induced deficits in the novel object recognition test and spontaneous alternation task, demonstrating a causal link between the transmitter switch and the cognitive deficits. Interestingly, chemogenetic stimulation of parvalbumin-positive inhibitory interneurons that innervate pyramidal neurons, either during drug exposure, or later after the transmitters

have switched, suppresses pyramidal neuron hyperactivity and prevents, or reverses, the appearance of both the switch and the cognitive deficits (Pratelli & Spitzer, 2021).

A third study tests the hypothesis that transmitter switching contributes to the generation of autism spectrum disorders. Pregnant female mice are treated with either Poly Inosine:Cytosine (Poly I:C) or valproic acid (VPA) on embryonic day 12.5. In adulthood, the progeny exhibit enhanced stereotypy and deficits in social interaction. We identify a transient decrease in the number of GABAergic neurons and a matching transient increase in the number of glutamatergic neurons in the medial prefrontal cortex, at postnatal day 10, specifically in interneurons expressing parvalbumin or cholecystokinin. Genetic marking of the originally GABAergic neurons demonstrates that they acquire glutamate and confirms that the transmitter switch has occurred. The switch reverses spontaneously by one month. To test the role of the transmitter switch in the emergence of the behavioral disorders, we restore GABA expression specifically in postnatal parvalbumin and cholecystokinin neurons. This treatment prevents the gain of glutamate in these neurons and reverses altered behavior that otherwise would be observed in male mice (Godavarthi & Spitzer, 2020).

Additionally, we are continuing studies of the benefits that may be achieved through transmitter switching. One week of running induces an NPY-to-glutamate switch in neurons in the hilus of the dentate gyrus of adult mice, in addition to the acetylcholine-to-GABA switch that we found in the midbrain, as discussed earlier. The increase in the number of glutamatergic neurons induced by running matches the decrease in the number of neurons that express NPY and occurs in the absence of neurogenesis or apoptosis in the hilus. The switch is associated with improved performance of adult mice on tests of novel object recognition and contextual fear conditioning. We hypothesize that replacement of NPY with glutamate in hilar neurons reverses the inhibitory regulation that these neurons otherwise exert on the lateral entorhinal cortex-to-dentate gyrus projection. This substitution of excitation for inhibition may underlie the enhanced performance induced by one week of running (Zambetti et al., 2017).

Looking Backward and Going Forward

The road from embryonic development of electrical excitability to neurotransmitter switching in the adult brain has been exciting. There have been slow stretches and fast tracks, as well as intriguing side streets not discussed here. The trip was made possible by collaboration with wonderful colleagues (see Table 1). Our analysis of the development of electrical excitability was born out of a keen interest in learning the process by which it occurs. Curiosity-driven research once again paid off handsomely, providing the deep satisfaction of understanding the process quantitatively. Usefully,

Table 1. Collaboration with Students and Postdocs

Students	Postdocs
Paola Baccaglini	Janet Lamborghini
Alan Willard	Corey Goodman
Matt Lee	John Bixby
David Zimmerman	Leslie Henderson
Marsha Revenaugh	Angie Ribera
Leslie Blair	Greg Harris
Amanda Iles	Janet Holliday
Diane O'Dowd	Michel Desarmenien
Rosario de Baca	Shawn Lockery
Nicolas Hussy	Beverly Clendening
Keith Allen	Xiaonan Gu
Maureen Ruchhoeft	Uwe Ernsberger
Eric Olson	Jeffrey Rohrbough
Kate Ribbeck	Devorah Gurantz
Donald Hagler	Mike Ferrari
Steve Watt	Evanna Gleason
Nate Lautermilch	Tim Gomez
Guo-li Ming	Anne Vincent
Ronald Swanger	Paul Kingston
Yuliya Gorbunova	Tim Manning
Cory Root	Ray Smith
Gabriela Montsalve	Laura Borodinsky
Sharon Sann	Brent Miller
Julia Cronin	Matt Conklin
Margaret Lin	Davide Dulcis
Elena Minakova	Kurt Marek
Estuardo Robles	Michaël Demarque
Linda Chang	Xavier Nicol
Lisa Kurtz	Paola Plazas
Sera Chung	Sheila Rosenberg
Qian Xiao	Claudia Alvarez-Baron
Lin Xu	Dena Weinberger
Norma Velázquez-Ulloa	Alicia Güemez-Gamboa
Kwan Pyo Hong	Matt Eckler
Pouya Jamshidi	Hui-quan Li
Amy Guzdar	Stefania Zambetti
Vaishnavi Rao	Swetha Godavarthi
Da Meng	Vincent Luczak
Nandu Prakash	Marta Pratelli
Christiana Stark	Kyle Jackson
	Wuji Jiang
	Arth Thaker

it provided a pathway to the discovery of neurotransmitter switching in the adult mammal (Spitzer, 1979, 2017).

Three aspects of transmitter switching are particularly noteworthy. The first is that switching is most often observed between glutamate and GABA or vice versa. This might have been anticipated because these transmitters

are abundantly expressed in nervous systems. Additionally, the switch from glutamate to GABA is metabolically simple, involving only a single enzyme. The second point is that switching between glutamate and GABA, like other switches such as acetylcholine to GABA, appears to change the sign of the synapse from excitatory to inhibitory or vice versa. Switches in synaptic sign reverse the signals to downstream neurons. The third point is that transmitter switching causes changes in behavior in all cases in which behavior has been tested. Strikingly, behavior is changed even when the switch is between two inhibitory transmitters, such as dopamine and somatostatin. Thus, the impact of transmitter switching on brain function and behavior can be expected to be substantial, both for ill and for good.

There are several issues that I hope to address in the near future. One urgent question is whether there are morphological changes in the downstream (and upstream) connectivity of the neurons that have switched their transmitter. Particularly in the adult nervous system, where the process of transmitter switching takes several days, there is opportunity for branching or retraction of nerve terminals and the formation or loss of synaptic contacts. It is also important to examine the consequences of transmitter switching for synaptic physiology. At an early stage in the study of transmitter switching, I chose to jump over the synapse and leap to behavior to determine if switching had biological significance. Now it is time to fill in this important piece of the picture. It also will be interesting to learn whether a single transmitter switch can drive switching in downstream neurons and induce multiple concatenated, serial switches. I am particularly eager to find out, in cases in which transmitter switching has adverse consequences, whether transmitter switching can be overridden or reversed by noninvasive alterations in electrical activity. The ability of transcranial magnetic stimulation to affect spiking activity of neurons, in areas of cortex measuring less than 2 millimeters in diameter, may avoid problems associated with drug action on unwanted targets and reduce the cost of treatment. Overall, the prospects seem bright for manipulations of transmitter switching to treat mental illness and promote health.

Academic Life Beyond the Lab

My career got off to a strong start. Then, not long after I was promoted to professor, and had served on a variety of committees, I was elected chair of the Biology Department. Although this reduced my time in the lab, it was rewarding to solve problems for my colleagues and lead faculty recruitment and planning of student curriculum. Jennefer Collins, the superb management services officer, made the job easy. Fortunately, the position of chair is typically a three-year appointment on the UC San Diego campus, and I was soon back in the lab full time. A few years later, I had the rewarding opportunity to cochair the department with Peter Geiduschek, a brilliant

molecular biologist with a magnificent, wry sense of humor. I was chair in the morning. Peter was chair in the afternoon. This was a compromise acceptable to both of us because we were each immersed in our separate research programs. We did not see one another during the day, but talked on the phone each evening to provide mutual briefings. I recall those conversations with exceptional pleasure.

My experience as a department chair led to election as chair of the Academic Senate. Now the problems to be solved were those on the whole-campus scale. I enjoyed working with the administration, and particularly with Chancellor Dick Atkinson, to realize the benefits of shared governance that is one of the founding principles of the University of California. In contrast with decisions made solely by the administration, shared governance leads to decisions that integrate the interests of all parties. This partnership has been a major contributor to the success of UC San Diego. The collaboration requires a willingness to work together that has occasionally seemed in jeopardy. Later, when the Biology Department became the Division of Biological Sciences, I was elected founding chair of the Neurobiology Section. This was the most enjoyable administrative position yet, providing leadership for a smaller group of colleagues, all of whom were deeply invested in studies of the nervous system.

Looking back, I recognize that I have enjoyed starting enterprises that have, at least so far, gone on to thrive. There are several features that seem to fascinate me. There is the opportunity to develop and shape a vision of the possible future. There is the energy that comes with starting something new. There is the tension that failure could be lurking. The opportunity to launch the Kavli Institute for Brain and Mind (KIBM) was such an enterprise. UC San Diego Chancellor Bob Dynes had sat next to Fred Kavli at a meeting of the President's Council of Advisors on Science and Technology and had learned about the Kavli Institutes that Fred was planning to create. The institutes would be established under the auspices of the Kavli Foundation, with its substantial assets from his business success. Later, in 2003, Fred visited the campus to discuss the possibility of establishing an institute at UC San Diego. Jeffrey Elman, a professor of cognitive science well known for his contributions to the field of neural networks, and I were asked to assemble a proposal for consideration by the Kavli Foundation. We consulted with colleagues and presented a vision for an institute that would bridge disciplinary boundaries in order to understand human cognition, from the brain to the mind. The KIBM was one of the group of four institutes that Fred established first. Jeff and I greatly enjoyed working together. We established several programs, enabled by the founding endowment of $7.5 million. For eight years I was glad to play the role of codirector and then director for three years, when Jeff stepped up to become dean of the Division of Social Sciences. Rusty Gage joined me as codirector for another five years. Catalyzing new scientific research and bridging the gap

between neurobiology on the one hand and cognitive science and psychology on the other, the highly successful Innovative Research Grant program annually funded 10 seed grants at $50,000 each.

Another opportunity to launch a new enterprise came from the Society for Neuroscience. I had been a member ever since my arrival at UC San Diego, serving on several committees, and in 2000 was elected to the SfN Council. I found it stimulating to be involved in planning the activities of the Society and determining its future initiatives. Particularly interesting were discussions of the benefits and drawbacks of investing in the construction of what became the Society's office building at 1121 14th Street, NW. In 2011, I was invited to become the founding editor in chief of *BrainFacts.org*, the Society's public-facing website about the nervous system that aims to inform and educate a broad audience. UC San Diego is a public university, and I had been teaching neurobiology to undergraduates for four decades. Here was a chance to reach an even larger audience. It was a splendid team effort. Early on, the Kavli and Gatsby Foundations committed support. We had an outstanding editorial board of neuroscientists, the full involvement of the Public Education and Communication Committee that I had previously chaired, and a spectacular SfN staff. There were consultants for online surveys and for focus groups to assess interest, and consultants for web design. An absorbing, collegial energy pushed us all forward. It was rewarding both then and now to see the number of hits on the website grow and to see other Foundations jumping in to provide support. Since I stepped down as editor in chief in 2014, the reach and impact of *BrainFacts.org* have continued to expand.

Coda

I write this in what one hopes is the closing phase of the great coronavirus pandemic of 2020–2021. It began with a lockdown and complete stoppage of all university activity and confinement to home. We came to the lab for mouse and frog colony maintenance. Experiments slowed to a crawl. My lab kept in touch with weekly Zoom journal clubs and lab meetings. Data analysis began to catch up with experimental results. Figures were prepared. We wrote and published what we hoped was a preview of things we could learn about transmitter switching in the future, thinly disguised as a review article (Li et al., 2020). Gradually, we have been able to return to a semblance of business-as-usual, working masked and in shifts to ensure adequate distancing. My colleagues were amazing, pleasantly pushing to and beyond the limits of what the campus allowed. Nonetheless, the pandemic inserted a delay in our progress at a time when our pace was accelerating.

My family has been consistently supportive of my work. Janet Lamborghini and I had two children, Julian and Hilary. Xiaoping Wang, my second wife, and I had three children, William, Addison, and Alexandra.

I assisted with many homework assignments and attended many soccer games, but I am grateful to both Janet and Xiaoping for the lioness's share of the heavy lifting of childrearing. After a visit to the Hirshhorn Museum, during a Washington, D.C., study section meeting, I built Julian a tensegrity jungle gym copy of Kenneth Snelson's Coronation Day sculpture. At the conclusion of another study section, Julian flew to Washington and we visited the Air and Space Museum and other engaging sights. Visits to the lab provided opportunities for Hilary, Addison, and Alexandra to see frog embryos and tadpoles under a microscope when not creating artwork on the whiteboard in my office. We enjoyed hiking in the Torrey Pines State Park and along the beach, climbing Mt. Woodson and Stonewall Peak in east San Diego County, and camping in the Anza Borrego Desert. Annual ski trips to Mammoth in the winter and vacations in Vermont in the summer were keenly anticipated. My family was understanding of my absence on trips for seminars and conferences. I took them with me whenever I could.

Thirty years ago, I began solo trips to the Sierra Nevada Mountains of California to climb the high peaks. I knew I enjoyed climbing from childhood trips with my parents. Life was marching on. If not now, when? I found that long weekends of solitude above 10,000 feet, with magnificent views, provided inspiration to think creatively about my scientific objectives. One Monday in 1995, UC San Diego Vice Chancellor Bruce Darling learned at Dick Atkinson's cabinet meeting that, although I was chair of the Academic Senate and usually in attendance, I was absent because I was in the hospital following a climbing accident. Bruce was an accomplished alpinist and had climbed all over the Sierras. As soon as I had recovered, we started rock climbing and ice climbing together. We also shared an enthusiasm for backcountry ski touring and launched an annual series of trans-Sierra ski tours, crossing east-to-west or north-to-south over the course of a week. We took friends when we could persuade them to join us, and have made 13 of these trips during the years when the snowpack permitted. We hope that the warmer winters associated with climate change will not curtail these high adventures.

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