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STEPHEN W. KUFFLER

1913—1980

A Biographical Memoir by

JOHN G. NICHOLLS

Biographical Memoir

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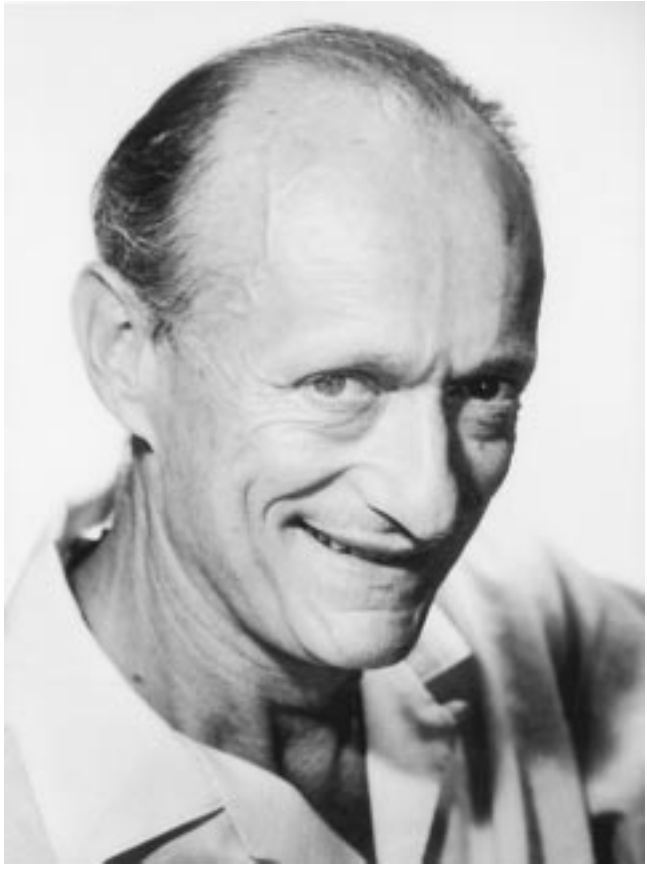

STEPHEN W. KUFFLER

August 24, 1913 – October 11, 1980

BY JOHN G. NICHOLLS

FROM THE BEGINNING of Stephen Kuffler's career in neurobiology until his last experiments, each paper he produced was distinctive for its clarity, elegance, and originality. Time after time he provided fresh insights into mechanisms by which nerve cells generate electrical impulses, transfer information at synapses, and integrate signals. A hallmark of his work was that after formulating a key question, Stephen Kuffler would seek and find just the right animal species and the appropriate techniques for obtaining a decisive answer. Although he tackled a wide range of fundamental problems, a continuous thread ran through his work: the desire to understand how neurons that make up the brain carry out their functions. To this end he made electrical recordings, often requiring hours of skilled dissection, to study the functional properties of individual nerve cells and muscle fibers in invertebrates, frogs, and mammals.

A characteristic feature of his experiments was the use of whatever electrical, biochemical, or morphological techniques were necessary for solving the problem. This approach produced a major change in the study of the nervous system. By virtue of his superb research, his personality, and the generations of students that he inspired and influenced, he

was an undisputed leader and dominant figure in neurobiology. To all his friends, colleagues, and students he was known as Steve.

EDUCATION AND EARLY LIFE

Stephen Kuffler was born on August 24, 1913, in Tap, a village in Hungary. His father, Wilhelm Kuffler, was a landowner living on a large estate. After his mother died when he was five years old, Steve was brought up by governesses at home until he went to a Jesuit boarding school in Austria at the age of ten, where he stayed until 1932. Steve often spoke to me about his childhood and youth. He was particularly happy at home where he had the free run of the estate; he greatly enjoyed horseback riding.

At school Steve studied humanities, Latin, and Greek, but virtually no science. In 1932 he went to medical school in Vienna; while he was a student his circumstances changed drastically when the family fortune was lost. The suddenness of this change from affluence to financial hardship had a profound effect on his view of life. During his training in medicine (which he was able to continue, albeit under straitened circumstances), he visited Egypt and England, which he enjoyed, except for a brief stint at the German Hospital in London. Steve found the atmosphere there to be authoritarian, repugnant, and reminiscent of the political atmosphere in Vienna with the growing intolerance and brutality that accompanied Nazism. After finishing his medical examinations in 1937, he worked in the Department of Pathology. Steve's distress at the situation after the *Anschluss* came to a head when he found that he had to do a postmortem on a colleague of his who had been murdered by the Nazis. After spending a few months in England he went to Australia, and it was there that his life as an experimen-

tal scientist began, through a meeting with Jack Eccles (Sir John Eccles, future Nobel Prize winner) on a tennis court.

PROFESSIONAL CAREER

The key catalytic event in Steve's scientific development was the arrival of Bernard Katz (later Sir Bernard Katz, Nobel Prize winner) who came to Eccles's lab in late 1939. From the very beginning Steve formed a close and long lasting friendship with Bernard Katz. Although Steve was to develop his own highly characteristic style of experimental research, Bernard Katz remained as the neuroscientist who most influenced his standards for the conduct of scientific research. In Eccles's lab Steve on his own made his first experiments on isolated nerve muscle junctions, which Bernard Katz described as "a brilliant technical feat . . . [that] . . . immediately and deservedly put him on the map." After the war Ralph Gerard offered Steve a position at the University of Chicago, where he worked for fifteen months before moving to the Wilmer Institute of Ophthalmology at the Johns Hopkins University Medical School as an associate professor and later professor. In addition to doing his own research he recruited a group of brilliant, independent young scientists, including David Hubel, Torsten Wiesel, Edwin Furshpan, and David Potter, together with an outstanding electronics engineer, Robert Bosler, with whom he was to work closely for the rest of his life. Steve also began to spend summers at the Marine Biological Laboratory at Woods Hole with his family and co-workers and started the first experimental lab courses devoted to the nervous system (the "Nerve-Muscle Program," later to become the neurobiology course). These intense lab and discussion courses had immense influence on generations of young graduate students and postdoctoral fellows coming from a variety of disciplines.

In 1959 the entire laboratory moved to the Department of Pharmacology at Harvard Medical School at the invitation of Prof. Otto Krayer, who offered generous space and facilities. At Harvard, Steve recruited a young biochemist, Edward Kravitz. A major contribution to the study of the nervous system was Steve's innovative idea of combining physiology, biochemistry, histology, neuroanatomy, and electron microscopy in one single group. In this way he shifted the focus of research from techniques that had been located in separate departments in universities throughout the world to neurobiology, a concept that Steve invented. From the time that the Department of Neurobiology was created in 1966 with Steve as chairman, he continued until his death to work in the lab with one or two postdoctoral fellows. Summers were spent at Woods Hole, except for the years 1967 to 1971, which were spent at the Salk Institute in La Jolla. Throughout his career, Steve provided the impetus for much of the research by his co-workers and criticized their papers in a light but decisive, inimitable style. Steve's name, however, appeared as author only on those papers in which he had done the experiments with his own hands.

MAJOR RESEARCH CONTRIBUTIONS

In the following paragraphs I summarize briefly highlights of Steve's research in roughly chronological order.

SYNAPTIC TRANSMISSION—FIRST STUDIES

Steve's style of research from the outset was to locate the Gordian knot and then cut right through it. By dissecting a single skeletal muscle fiber together with its nerve—an immensely difficult task—Steve could analyze the events occurring at the synapse with greater precision than had hitherto been possible in intact muscles. At a time when

intracellular microelectrodes had not yet been invented, his Australia papers on the properties of the end plate potential, on the effects of calcium, and on the changes produced by denervation set new standards for investigating synapses. As a student, I well remember reading each new paper with excitement and admiration. Other experiments with Bernard Katz on crustacean muscles set the stage for later important studies on inhibition.

SLOW MUSCLE FIBERS AND MUSCLE SPINDLES

Steve's initial work in Chicago was on slowly contracting muscle fibers in the frog and this in turn led him to the study of the sensory innervation of mammalian muscle. Although important pioneering studies had been made on sensory muscle spindles by B. H. C. Matthews in the early 1930s and by L. Leksell in the mid-1940s, the literature about the efferent output from the spinal cord to the spindle was abundant but confused and largely incomprehensible. (This was the usual starting point for Steve's generation of a new idea.) At Hopkins, together with Peter Quilliam and Cuy Hunt with whom he was to develop a close friendship and work for several years, he devised an elegant and direct experiment. Electrical recordings were made from a single sensory fiber coming from a muscle spindle receptor in muscle. At the same time an individual motor nerve fiber was stimulated. A large fiber, as expected, caused muscle contractions. When a single small diameter motor fiber was stimulated there was no overt contraction of the muscle, but the stimuli dramatically increased the frequency of the sensory discharge. This was due to activation of small specialized muscle fibers in the muscle spindle. In a series of elegant papers Cuy Hunt and Steve explored in detail the role of this efferent control by the nervous system of the information coming to it.

RETINAL GANGLION CELLS

In the next series of experiments at Hopkins, Steve turned to signaling in the mammalian retina. In 1952 it was impossible to understand the meaning of signals traveling from the eye to the brain. This was in large part because bright flashes of diffuse white or colored light had been used as stimuli. Through the invention with his friend S. A. Talbot of a new ophthalmoscope, Steve was able to stimulate well-defined discrete areas of retina by small, light, or dark spots. Once again in one series of experiments in which he was sole author, Steve revealed a fundamental mechanism. A key feature was to use natural stimuli to define the receptive field properties of individual ganglion cells and their optic nerve fibers. The major conclusion was that these cells responded primarily to contrast and to moving stimuli rather than diffuse light. These properties in turn depended on the convergence of excitatory and inhibitory inputs arising from cells in preceding layers of the retina.

A story Steve told me shows the impact of these retina papers. Steve had just presented his new findings at a meeting in Cambridge. Lord Adrian, the pioneer in our understanding of sensory signaling whom Steve greatly admired but had never met, was walking along a corridor from the other direction. As he encountered Steve he stopped, cocked his head, and asked simply, "Are they the same in the brain?" David Hubel and Torsten Wiesel have given fascinating descriptions of Steve and the way experiments he made on the retina provided the starting point for their own work in the visual cortex.

EXCITATION AND INHIBITION OF CRUSTACEAN STRETCH RECEPTOR

With Carlos Eyzaguirre, Steve made the most elegant and detailed study of the way signals are initiated in mechanoreceptors. He chose the crustacean receptor as the ideal

preparation because it could be isolated and explored with microelectrodes. In beautifully clear recordings they defined the properties of the generator potential, the essential intermediary signal between stimulus and conducted action potentials. In the same preparation they provided new insights into inhibitory mechanisms, again demonstrating efferent control by the central nervous system of information coming to it. An important pointer to the future was the study by Steve with Charles Edwards of the effect of gamma-aminobutyric acid (GABA), which mimicked the action of inhibitory nerves.

DEMONSTRATION OF GABA AS AN INHIBITORY TRANSMITTER

While there were some hints that GABA could mediate inhibition, Steve, Ed Kravitz, David Potter, and their colleagues provided the first definitive proof in lobsters. Comparisons of the actions of GABA with those of the naturally released transmitter revealed a close similarity. In back-breaking experiments, meters (literally!) of single inhibitory and single excitatory axons were dissected day after day from giant lobsters (a fringe benefit for people like me, who joined the lab at this time, were the lobster feasts). Biochemical analysis showed that inhibitory axons contained high concentrations of GABA, approximately a thousand times more than the excitatory axons. These experiments laid the foundation for subsequent work on GABA mechanisms in mammalian brain.

PRESYNAPTIC INHIBITION

Immediately preceding these GABA experiments Steve together with Josef Dudel had broken new ground by unequivocally demonstrating the mechanism of presynaptic inhibition, hitherto a somewhat ill-defined concept. By picking the right preparation, the nerve muscle junction in crusta-

ceans, it was possible to demonstrate that inhibitory nerves acted in an entirely novel manner. In addition to an inhibitory action on the postsynaptic muscle fiber, impulses in the inhibitory nerve reduced the amount of transmitter released from the excitatory nerve by impulses. Once again, a decisive series of experiments with far-reaching consequences.

PHYSIOLOGY OF GLIAL CELLS

By the time I arrived in the laboratory in 1962, Steve and David Potter had already chosen the ideal preparation for studying glia, the central nervous system of the leech. I remember my own initial amazement that anybody would want to study these cells, which were then considered to be the inert connective tissue of the brain. What Steve set out to do was to study their membrane properties and see how they compared to nerve cells. In leech ganglia Steve and David Potter showed that glial cells had higher resting potentials than nerve cells, were electrically coupled, and could not give impulses. Steve and I then went on to determine whether ions and small molecules reached the nerve cells from the vasculature by way of extracellular spaces or through the glial cells. Our results showed that narrow 250-Å extracellular clefts, not glia, acted as the pathway. With Dick Orkand we then used the optic nerves of frogs and mudpuppies to show that the properties of glial cells there resembled those in the leech. We also found a novel interaction: impulses in axons caused potassium to accumulate in extracellular spaces and thereby give rise to a glial depolarization. From this finding came the concept of spatial buffering whereby glial cells could control the extracellular environment of the neurons they surround.

Later experiments by Steve with Monroe Cohen and Hersch Gerschenfeld revealed key properties of the blood brain barrier.

SYNAPTIC TRANSMISSION

For the remaining years Steve returned to the study of synaptic transmission, particularly with U. J. McMahan, his close friend and colleague. Their motivation was similar to that of Bernard Katz (although the approach was very different): to understand in detail and quantitatively how nerve cells communicate at synapses. Jack McMahan and Steve took advantage of newly developed optical techniques (differential interference contrast) to observe living synapses between parasympathetic nerve cells in an ideal preparation (again!), the thin transparent septum of the frog heart. Here they and colleagues defined the structure of the synapses at the light and electron microscopic level and demonstrated that nerve-to-nerve synapses resemble physiologically those at the neuromuscular junction. Moreover, as in muscle, acetylcholine receptors spread to cover the surface of the cell after denervation. In other studies on autonomic ganglia with Doju Yoshikami and later with Lily and Yu Nung Jan, Steve made experiments that clarified what was then a confusing and chaotic problem. Considerable heated controversy existed about the properties of slow synaptic potentials in autonomic ganglia and the mechanism by which they arise. Through a combination of pharmacological, biochemical, and electrophysiological approaches, these slow excitatory and inhibitory potentials, which lasted for minutes or hours, were shown to depend in part on actions of acetylcholine on muscarinic as well as nicotinic receptors. In addition they provided the first unequivocal evidence for the release of a peptide (LHRH) from preganglionic terminals and its role in synaptic transmission.

Preceding these studies on ganglia, Steve and Doju Yoshikami published a pivotal paper on synaptic transmission at the nerve muscle junction. In exceedingly difficult experiments they measured the number of acetylcholine

molecules in a quantum, the unit of release from motor nerve terminals. The principle was to apply known concentrations of the transmitter in a highly localized manner instantaneously to the receptors at the motor endplate. By comparing quantitatively the artificially and naturally evoked quanta a direct estimate was made of the number of acetylcholine molecules. This was approximately 5,000, a concentration that could be achieved in a single synaptic vesicle.

What made Stephen Kuffler's papers so remarkable was that the point was never in doubt. Even today it is easy to see how each research project decisively took on an issue that was messy, occult, undecided—or not even thought of—and brought it to a new level of understanding. The earlier papers, like the later ones, are easy to read, economical, and written with flair. The clarity of the thinking and the presentation as well as the direct answers usually obviated the need for long discussions.

STEVE IN THE LABORATORY

No one had greater disdain than Steve did for sloppy thinking or sloppy experiments. Yet this attitude was never translated into unkindness at the personal level. A somewhat sharp but subtle wit was his instrument for deflating pomposity or countering aggression. During experiments he *worked;* you would try again and again and again, all day and late at night, and again the next day until you got good recordings you could rely on. Single mindedness and dedication during experiments were in contrast to the relaxed, vague, almost amorphous approach with which long-term projects were discussed in the first place. He used to say that it was silly to do experiments that could take weeks or months without spending a decent time discussing what to do. I believe that he worked by thinking at great length about what was the most interesting project he could solve (that he could undertake himself with his own particular

talents). Then came the enjoyment of finding the right preparation. A new project was a time of constant exploration, feeling out different technical approaches, and continually redefining one's objectives. Much the same was true of the way we wrote a book together (*From Neuron to Brain)* every summer for seven years or so. No amount of time was too long to devote to the title (I think we spent three weeks on that), the table of contents, the structure of a chapter, or the esthetics of the figures. At the same time a feature of experiments made with Steve that made them such unending pleasure was the series of jokes, comments, banter, and reminiscences of colleagues. The jokes would flow freely with improvisations, puns, and set-piece jokes. Through his talking with such affection about his previous co-workers, one got to know them. Thus, long before I had met Cuy Hunt or Werner Loewenstein, I looked on them as friends; the same was true even of people who had died, like Joe Lillienthal and Julian Tobias, who had been close friends of Steve's. I never knew him to make a malicious joke or a joke at someone else's expense.

The number of deep and long lasting friendships Steve formed with students and colleagues greatly exceeds the few names mentioned above. The general feeling of excitement in the charmed circle of Steve's department was due to the brilliant students, the extraordinary research being done by the young faculty, and by the infrastructure provided by Marion Kozodoy, Steve's secretary and administrator. None of us ever needed to waste hours of time that could be devoted to experiments on administrative details, which she and Steve somehow handled.

HONORS AND AWARDS

Steve was widely recognized as a truly original and creative neuroscientist. In addition to numerous prizes, honorary degrees, and special lectureships from countries over

the world, Steve was elected to the National Academy of Sciences in 1964 and to the Royal Society as Foreign Member in 1971. In 1964 he was named the Robert Winthrop professor of neurophysiology and neuropharmacology. From 1966 to 1974 he was the Robert Winthrop professor of neurobiology, and in 1974 he became John Franklin Enders university professor.

FAMILY

Steve and Phyllis (née Shewcroft) were married in Australia in 1943. She had attended his medical school lectures in physiology. In addition to being a doctor, she was an accomplished painter and educator and received a doctorate from Harvard University. Their oldest daughter Suzanne is a painter. Damien is a well-known neuroscientist in his own right at the University of Puerto Rico. Eugenie is a composer, flautist, and performer in Paris and Julian is a physician in Maine. The four children, Phyllis, and Steve provided warm hospitality and friendship to Steve's "scientific family" from around the world. Towards the end of his life he suffered from diabetes and glaucoma. Nevertheless, he continued to work, swim, travel, and play tennis with uncanny, cat-like ability, enjoying life at home and in the lab to the end. Only his closest friends were aware of the drastic deterioration of his vision or the precariousness of his insulin treatment.

CONCLUSION

Steve's importance for neurobiology was unique. His imaginative experiments have stood the test of time and provided essential pointers for others to take up where he left off. Numerous distinguished molecular biologists and geneticists, such as Gunther Stent and Seymour Benzer to name just two, were attracted to neurobiology by his work.

His immense influence as a teacher was not due to assertiveness or rhetoric but to example. He maintained the highest standards in his students and co-workers by a quizzical look, a mild quip, or—worst of all—boredom. I was always curious about how his philosophy and code of behavior had developed. Coupled with a hatred of extremism, he showed endless sensitivity and consideration in dealing with other people in every walk of life. The only sign I saw of a double standard in his conduct was the contrast between his own lack of consideration for himself and the infinite trouble he would go to for colleagues who were in need of help. It requires poetry or art rather than a standard obituary to convey Steve's joie de vivre, his love of experiments, his love of friends and family, his patience, tolerance, enthusiasm, wit, and wisdom.

A DETAILED, AFFECTIONATE, and authoritative account of Stephen Kuffler's life and work has been provided by Sir Bernard Katz (*Biographical Memoirs of Fellows of the Royal Society*, vol. 28, pp. 225-59, 1982) and in a book entitled *Steve, Remembrances of Stephen W. Kuffler*, compiled and introduced by U. J. McMahan (Sunderland, Mass.: Sinauer Associates, 1990).

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