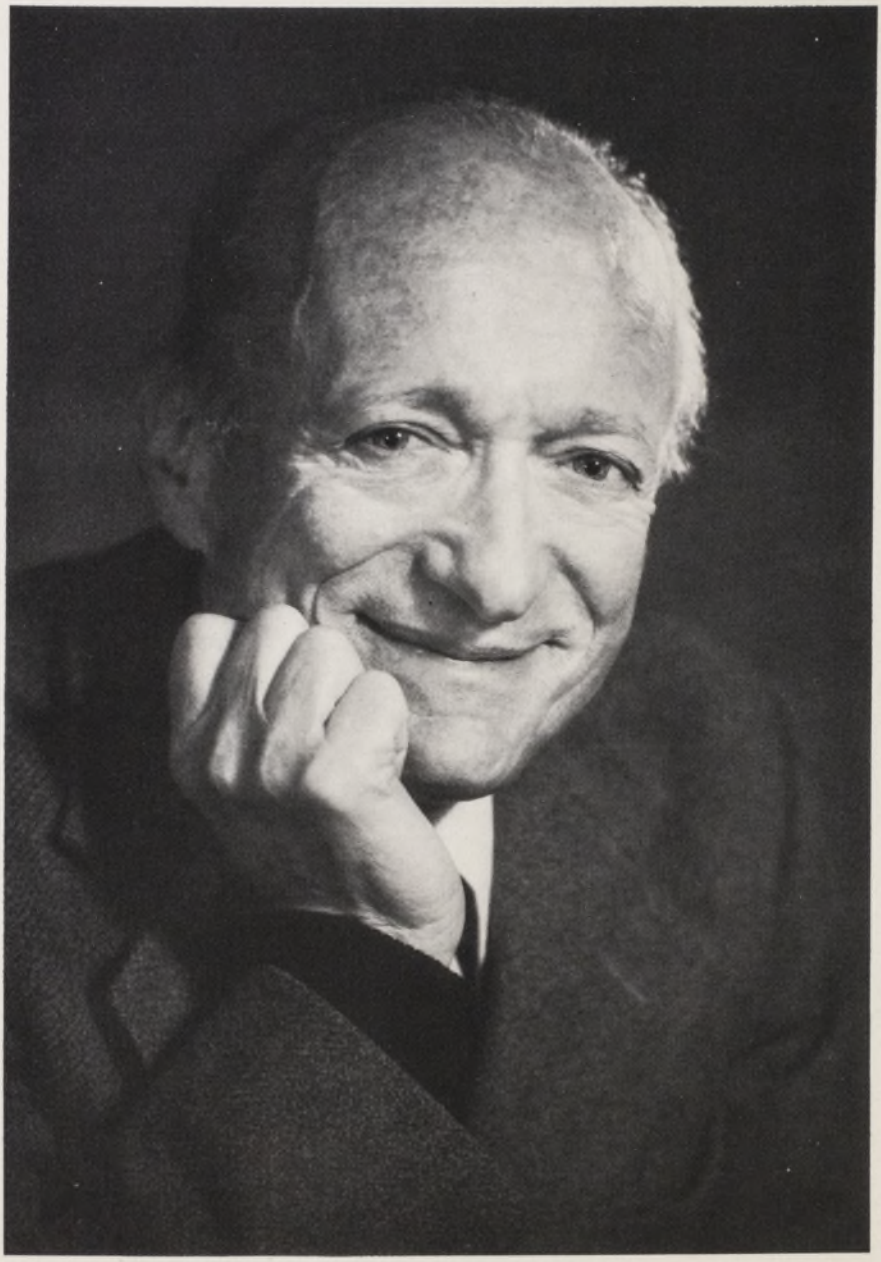

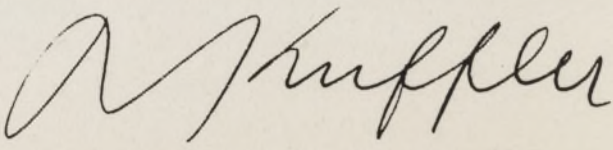

STEPHEN WILLIAM KUFFLER

24 August 1913 — 11 October 1980

Elected For.Mem.R.S. 1971

By Sir Bernard Katz, F.R.S.

Contents

STEPHEN KUFFLER who died at his home in Woods Hole, Massachusetts, at the age of 67, was much beloved and admired, as a scientist and as a personal friend, by colleagues and pupils all over the world. He was acknowledged as one of the leading neurophysiologists of his generation whose work illuminated many different aspects of our nervous system and who by his brilliant experimental skill often achieved results that gave aesthetic as well as intellectual satisfaction. He died suddenly, at the height of his scientific activities. Though his close friends had warnings for many years of his precarious state of health, he had borne serious illness, glaucoma, cataract, several eye operations, diabetes, heart trouble, without complaint, with no sign of losing his cheerful disposition or his seemingly flippant sense of humour, and certainly without letting it interfere with his great pleasure in making experiments or even with his athletic hobbies, swimming and tennis.

ORIGIN AND CHILDHOOD

He left few notes about his early life (see Appendix II) and much of the following is compiled from information I received from his family and friends.

He was born on a country estate in Hungary where he spent the first 10 years of his life. His birth certificate describes him as Wilhelm (or Vilmos in Hungarian) Kuffler, born 24 August 1913 at the village of Táp, son of Wilhelm Kuffler, landholder, 27 years and Elsa Kertész, 25 years. Parents and son belonged to the reformed (Calvinist) church; this was quite common among the Hungarian landed gentry, who after the Reformation had turned Protestant to demonstrate a degree of independence from the Catholic Habsburg dynasty.

These entries in his birth certificate differ in a curious way from what we knew of him, and there are certain ambiguities about his choice of first name, his nationality and even his religious denomination. He evidently did not care much for 'Wilhelm' or William and preferred the name Stephen which he adopted in 1938 at the time of his emigration from Austria. It is not quite clear whether to consider Stephen as Hungarian or Austrian by origin, and he would probably have regarded the question as irrelevant in any case. It appears that, when the Habsburg empire was dismantled in 1919, Stephen's family opted for Austrian nationality. Later on, after his emigration, he became a 'stateless' person until he acquired British (Australian), and, finally, United States citizenship. As to his religious affiliation, he was brought up in the Roman Catholic faith, and was probably not aware himself of his Calvinist origin until he happened to examine his birth certificate in adult life. I do not know whether Stephen was ever a deeply religious person; my own impression was that his attitude towards the demands of his church was one of peaceful acquiescence rather than strong conviction, and even this may

have diminished as he became more fully absorbed by science. But there is no doubt that his Catholic connections had a most important influence on his life and the direction of his academic career, especially through the social contacts and personal associations they brought him after he left his native country, in particular meeting Jack Eccles in Sydney who gave him a start in physiology in 1938, and Phyllis Shewcroft whom he married in 1943.

In a brief biographical essay, Stephen speaks about his early life. 'I spent the first ten years of my life in Hungary on a medium-sized farm. My most vivid memories are about riding horses, swimming in ponds and occasionally visiting a nearby "big" city. At home we spoke Hungarian and German and learned reasonably good French from a succession of governesses.' The description of his home is probably an understatement. According to other accounts, it was quite a large estate with most of the villagers being employed on it; it housed a beautiful mansion surrounded by a magnificent park, replete with stables, ponies and grooms for Stephen and his two sisters, Elizabeth and Margaret. It was an idyllic existence which continued even after the tragic death of his mother from typhoid fever in 1918. The father (who was the eldest of six brothers) was running the estate as well as owning valuable property in Austria and in the capital, Vienna; he was an agricultural expert who had studied and obtained his academic diploma at the School of Agriculture in Berlin. After the mother's death, an Austrian lady together with a younger German assistant were engaged to look after the family; this arrangement worked very satisfactorily for the next few years until the father remarried and his new wife, Margit, a young widow with two daughters, took over the running of the home. The family life continued to be very happy, with 'nice memories of huge Christmas trees, mountains of toys and outings on horseback' (Margaret Wilmot's account). The final addition to the family was another girl, Marian, a half-sister to Stephen.

School

The idyllic country life came to a final and sudden end in 1923 when Stephen was sent to a boarding school at Kalksburg outside Vienna, a classical type of 'gymnasium' run by Jesuits, which enjoyed a high reputation both for its scholastic attainments and for the humane and fairly liberal spirit which prevailed in spite of the strictly conservative religious tradition. This was, however, not the first traumatic interruption in Stephen's early life style. In the summer of 1919, during the short-lived communist regime of Béla Kun, the family had to make a quick escape across the Austro-Hungarian border, and I am told that the 6-year-old boy experienced a gun being pressed in his neck by some commissar who interrogated him about his father, who was already in Austria. It must have taught him a lesson which he put to use 19 years

later, when he decided not to delay his departure (that time from Austria to Hungary)!

At Kalksburg, Stephen found himself in an entirely new environment and at first without the necessary preparation. 'A few attempts in elementary education on a private basis by the local school teacher [at Táp] had ended in complete failure.' The teachers at the boarding school decided very wisely to give Stephen a year of preliminary education to let him make up for his missing elementary schooling. After that, he took the full 8-year course which consisted of much Latin and Greek 'and practically no Science'. From accounts of one of his schoolmates, he was good, but not outstanding, did well in academic subjects and also at his favourite sports, swimming and tennis. He was popular and well-liked by his contemporaries, but apparently showed a certain restraint in his human relations which prevented the formation of close friendships.

University

When Stephen left Kalksburg in 1932, his training at the gymnasium with its emphasis on linguistic studies had given him a bias towards the humanities or law. Nevertheless, for practical reasons and because of its international application he decided to go in for medicine at the University of Vienna. His progress is probably best described in his own words (see Appendix II), to which I add a few annotations. The University entrance requirements at that time, in Germany and Austria, were very simple: anyone with an 'Abitur' (high school leaving examination) had the right to enter the Faculty of his choice at any University, and one could also switch courses from one academic institution to another. Students who took up medicine or science, but came from a traditional type of classical 'gymnasium', received their basic scientific training in the first 2 years at the University. It was a matter of luck whether this training was inspiring and given by a teacher of great scientific achievements or by someone who regarded his lectures as an undesirable chore; in any case the student had much freedom of choice and could decide for himself which courses he wished to attend. There could be great advantages in being taught elementary science, for the first time, by an outstanding University teacher (I recall, as a junior medical student coming from a 'humanistic' school, receiving a memorable course of lectures and demonstrations in basic physics, given by P. Debye every day from 8 to 9 a.m.); but Stephen evidently did not have this kind of experience and, from his account, he seems to have suffered from a lasting feeling of inferiority in scientific and theoretical matters. But I was surprised to read of the 'almost unbearable challenge' of working with his seemingly more sophisticated colleagues (Appendix II); our working relations, at the time he refers to, were close and of the most friendly kind, with our daily experiments being accompanied by hilarious banter

and almost competitive high-speed production of jokes and atrocious puns!

The year after Stephen started on his medical course, his father suffered a catastrophic financial reverse, and from then was unable to support him. Soon afterwards, the father died as a result of a stroke. Stephen received some help from his uncle Paul Kuffler, which enabled him to save enough for his travels abroad, but he had to finance himself by tutoring high-school boys and generally live with utmost economy.

For a time he left the students' hostel in Vienna and gave up his membership of a catholic students' society to take up residence in the house of his employer outside Vienna, as a family tutor. Even so, he managed to spend a summer vacation in the German Hospital in London and to hitch-hike to Egypt where his sister Elizabeth was working as a dentist in Cairo and where he also made the first acquaintance of the author John Brophy with whose family he formed very close ties.

In his final year at the University he rushed through his twelve clinical examinations, finishing them in record time and obtaining his M.D. in December 1937. Thereafter he worked for 3 months as an unpaid assistant at the 2nd Medical University Clinic and at the same time in the Institute of Pathology. As an undergraduate, pathology had been his favourite subject; for physiology he had no liking at all!

In his personal notes Stephen tells us some of the reasons which made him leave Austria after the 'Anschluss'. I understand there were additional, and probably more compelling factors, and I have taken the opportunity of discussing them with his closest colleague among his fellow students. He had been involved, though not very actively, with a conservative anti-Nazi group of students who had been planning some form of resistance against the Hitler invasion. They even possessed a small quantity of arms, but as with many of the other 'paramilitary' formations, it came to nothing, the police confiscated the weapons one day after the German troops had invaded Austria, and fortunately a sympathetic police officer destroyed the membership list which had come into his possession. Stephen was not aware of this last, very important circumstance, and, having been asked to report to the police the next day, he decided to make his getaway immediately. He had no difficulties in crossing the Hungarian border, as he had the necessary papers enabling him to make periodic visits to his old home. From Hungary he proceeded to England via Trieste, and after 3 months earning some money as a kind of companion (cum-teacher-cum-chauffeur) at a country mansion near Manchester and spending some time with his great friends the Brophys in London, he went by boat to Australia.

It appears that he greatly resented having to leave Vienna, and although many of his friends and acquaintances suffered political persecution under the Nazis, political reasons were probably not the primary motive for his emigration. His paternal grandmother came from a Jewish

family, and he of course realized that this was quite enough to make life most unpleasant, if not impossible, under the new rulers of Austria. His uncle Paul who had helped him during the difficult undergraduate period was forced into labour service by the Nazis and died during the war.

Australia

Stephen Kuffler arrived in Sydney in the summer of 1938. He met Professor Keith Inglis who had recently been appointed Head of the Pathology Department at Sydney University. (Before then, Inglis had been the first Director of the new Kanematsu Memorial Institute of Pathology at Sydney Hospital, and in 1937 was succeeded in this post by Dr J. C. Eccles, the well-known neurophysiologist—former Rhodes scholar from Melbourne who had worked with Sherrington at Oxford for many years before returning to Australia.) Inglis offered Kuffler an appointment as a demonstrator in Pathology, but it was an unpaid job, and his savings must have been dwindling at an alarming rate. He kept it up for 2 weeks and had the great fortune of being introduced, by a Jesuit priest Fr Richard Murphy, to Jack Eccles who, at the time, was looking for somebody who could give him a regular good game of tennis, and perhaps also make himself useful in the laboratory. Stephen seemed to have the right qualifications, at least for the former role.

Although Eccles had been appointed officially as Head of a Pathology Institute at a large general hospital, it was understood that he would not be required to direct the routine pathological work, and he was given a separate floor at the top of the building where he could establish a research laboratory in the field of his personal choice. He had support grants from the Australian Medical Research Council and was able to offer Stephen a junior post and encouraged him to join in his electrophysiological experiments which at that time were concerned with neuromuscular transmission.

During the first 12 months, Stephen found the subject bewildering and not without reason, for the interpretations placed on some of the electrical records were as confusing as the accompanying terminology; entities had been postulated (the so-called 'detonator response') for which no visible evidence could be produced, and it took a few years to eradicate them from the literature. When I arrived on the scene, in 1939, I could sense that Stephen had not yet come to terms with physiology, and for another year or so, I was wondering whether he would make it his career. If I had any beneficial influence, it was probably my reluctance to join Eccles and Kuffler in their mammalian experiments. Their work was done entirely on *in situ* recording from the anaesthetized whole cat; Stephen was very good in setting up this type of preparation, while I was not and did not even like it. Bringing with me the tradition of A. V. Hill's laboratory where most experiments were done on isolated tissue prep-

arations of cold-blooded animals, I managed to persuade Stephen that there were certain advantages in working on simple systems, especially for the study of cellular and single synaptic processes. For a change, we introduced some nice Australian tree frogs (*Hyla aurea*) into the laboratory whose isolated nerve-sartorius preparations were just as suitable for long-lasting experiments as those of the English *Rana temporaria* to which I had been accustomed. I still remember Stephen roaring with laughter when I showed him how to 'take the frog's trousers off', a procedure which I mistakenly thought was known to every medical student. The laughs were on my side when I saw Stephen take his first sartorius muscle and place it into a beaker of hyposulphite instead of Ringer's solution. The real turning point in Stephen's career came a year later, in March or April 1941, when with Eccles's encouragement he pursued the isolation technique much further and produced the first work on a single isolated synapse; this will be described in the experimental section below. This was his first solo performance as a physiologist, and it immediately established his international reputation as a first-class experimenter. An article by Renshaw in the *Annual Review of Physiology* of 1943 already singles out Kuffler's 'beautiful experiments' on the isolated neuromuscular junction, and his series of papers in the *Journal of Neurophysiology* starting in 1942 were impressive enough for him to be appointed a National Research Council Fellow in Sydney (1943–45) and for R. W. Gerard to invite him in 1944 to take up a Seymour Coman Fellowship in Physiology at the University of Chicago.

Another event that was of importance for his subsequent career was the arrival in Sydney of Major A. M. Harvey, who had come with a group of medical officers from Baltimore to establish a field hospital for American soldiers in a Sydney suburb. Harvey's research interests, before he became Professor of Medicine at Johns Hopkins Medical School, had been in the field of neuromuscular transmission on which he had been working in Sir Henry Dale's laboratory in the 1930s. He re-established contact with Jack Eccles in Sydney, and this led to a fruitful collaboration with Stephen and to some joint papers on applied electrophysiology in humans. Stephen became an honorary part-time consultant with the U.S. Medical Corps during that period and, what was more important, A. M. Harvey got to know him well enough to arrange for Stephen to be offered a position at Johns Hopkins University in 1947.

Marriage and family

In April 1943 Stephen was married to Phyllis Shewcroft, who had just graduated from Sydney University Medical School. They had met first in April 1940, during some lectures which Eccles and I gave to the junior medical students in their Physiology course. Phyllis Kuffler, in addition to raising a family of four children, has had what one might call a multi-

disciplinary career: starting as an art student, then becoming a medical doctor and after their move to the United States acquiring expertise in educational psychology, with her particular interests in young persons' appreciation of visual arts and music. In these subjects she showed great enterprise, working with a youth orchestra and taking it on overseas tours, starting an art studio for young people, and teaching at the Rhode Island School of Design at Providence.

Their first child, Suzanne Elizabeth born in Sydney in 1945, is an artist currently working at Harvard Medical School. The other three were born in America, in 1947–49: Damien Paul, now a postdoctoral fellow in neurobiology at Stanford University; Julian Phillip, first teaching, then taking up medicine and now finishing his Internship at Maine Medical Center, and Eugenie Gabrielle who studied music in Paris and is active as a composer and performer.

Moving to the United States

In 1944, J. C. Eccles decided to leave, after some disagreement over policy questions with the administrators of Sydney Hospital, and to accept the Chair of Physiology at Otago University in New Zealand. Kuffler was able to continue his researches until 1945, but no prospects of a satisfactory appointment were in sight, and after obtaining his naturalization papers he applied for a United States visa to take up R. W. Gerard's invitation from Chicago University. This took some considerable time, and eventually an addendum had to be obtained to cover the newly arrived Suzanne as well as her parents. In the autumn of 1945, the Kuffler family finally departed by boat across the Pacific Ocean and settled for 15 months in Chicago. The initial period cannot have been easy, but it took Stephen very little time before he got down to some interesting work on the slow 'non-spiking' muscle fibres in the frog, and he quickly made friends with many American physiologists and with foreign postdoctoral visitors, in particular Yves Laporte from Toulouse (now at the Collège de France) who joined him under a French Government Research Fellowship in 1946. Stephen's move to Baltimore, to the Wilmer Institute of Ophthalmology at Johns Hopkins Medical School, is well described in his personal notes, where he refers to it as a 'wonderful place', with himself given charge of 'a small basement laboratory that soon became filled with a group of eager young postdoctoral workers, several of them from abroad, particularly Great Britain'. He stayed at Hopkins for 12 years, first as Associate Professor, later as Professor of 'Ophthalmic Physiology and Biophysics'. The first few years were a period of very close collaboration with C. C. Hunt, especially on muscle spindles and intrafusal motor innervation. Later came other important work, on the mammalian retina, more on nerve-muscle

transmission in 'slow' fibres and on the stretch receptor neurons in Crustacea—some of it done at Woods Hole.

Kuffler's association with the Marine Biological Laboratory started in 1947; for many years the whole family used to spend the summer months at Woods Hole, at first being housed in a cottage close to the class rooms where he taught physiology and initiated a neurobiology training programme. Later they bought a house a few minutes away, near to the beach on Buzzard's Bay. Over the years, Stephen and Phyllis greatly improved and enlarged it. Stephen loved this house; it became to him a kind of dream home, a place of peace and refuge to which he returned even during the winter months when the laboratory and the village were almost deserted. There was an intermediate period starting in 1967, when Stephen and Phyllis decided to spend the summer vacations at La Jolla in Southern California and he was doing his summer work at the Salk Institute, but after 1971 he returned to his favourite summer residence at Woods Hole.

In a letter written in February 1958 to a distant cousin in South Africa, Stephen summarizes his Baltimore cum Woods Hole years as a very happy period for the whole family 'even after eleven years of it'. Johns Hopkins University had given him 'a generous appointment as Professor with practically no teaching'. His job was to do research and graduate training. The 'routine is very simple, coming in the morning and staying as long as the experiments require'. But the weekends were spent at home. He ends by saying: 'I have practically no hobbies except the family and work, which sounds somewhat dull but is not.'

Early in 1956 he was approached by the University of Basel with the suggestion that he might succeed Professor Verzar who was retiring from the Chair of Physiology, but he was well settled in the United States and it is hardly surprising that he did not pursue the matter. This was not the only approach: in November 1958 the question was mooted whether he would be interested in succeeding W. O. Fenn in Physiology at Rochester, N.Y. Kuffler's reply was non-committal, he clearly did not want to chair a big department with heavy administrative and teaching responsibilities. Nevertheless, with his rapidly growing international stature, the requests from intending postdoctoral visitors and the pressure on laboratory space increased, so much that 'by the middle 1950s the place became unbelievably crowded with capable and productive young people. This state of affairs was noticed by Harvard Medical School and they offered us space and opportunities.'

Some of Stephen's colleagues at Hopkins felt that there would have been no great difficulty in giving him all the space and facilities he required had he asked for them. But apart from this, there may have been other attractions which induced him to make a move, such as the powerful concentration of scientific activity and of excellent students at Harvard and M.I.T. and, perhaps even more important, the proximity to

Woods Hole. Anyway, by 1958–59 he felt that Harvard Medical School, on the initiative of Professor Otto Krayer, were making him 'an offer which he could not refuse'. So, in June 1959, 'about ten of us migrated to Massachusetts'. They included Torsten Wiesel, David Hubel, Edwin Furshpan, David Potter and, last but not least, R. B. Bosler who looked after the technical needs with great efficiency and held himself responsible for maintaining and continuously improving the high-class electronic apparatus which was such a vital asset and indeed indispensable for the success of Stephen's experimental work. Shortly after their arrival in Boston they were joined by E. A. Kravitz.

For over 20 years, until his death in 1980, Stephen Kuffler remained at his post at Harvard Medical School. The names attached to his appointment, and the administrative duties associated with it, changed in the course of time. He had been invited by Otto Krayer to come as Professor of Neurophysiology and Neuropharmacology and thus to add a powerful group to the Department of Pharmacology which had already under Krayer's direction established itself as one of the leading institutions in the field. In 1964 the name of Mr Robert Winthrop, a well-known benefactor was added to the professorship, but Stephen and his group remained in the Department of Pharmacology until Otto Krayer's retirement. It was, in effect, a gradually spreading sub-department devoted to a study of the nervous system, with a strong tendency to become 'multidisciplinary', going beyond the electrophysiological approach and adding the techniques of biochemistry and electron microscopy to its arsenal. In 1966, Harvard Medical School decided to establish the group formally as a Department of Neurobiology with Stephen as Chairman. He kept this position for the next 8 years, but in 1974 decided to relinquish the administrative duties associated with the running of the increasingly large department and became John F. Enders University Professor, a position which enabled him to devote his efforts entirely to research without imposing any physical changes, in facilities or location on him. This was, of course, due in no small measure to the great personal support which he received from his friends, in particular from Torsten Wiesel who had taken on the administrative headship of the department in 1974.

The strength and influence of Kuffler's Neurobiology Department was recognized internationally, and not only by those who read the publications and who received the famous annual cartoons and Christmas cards which depicted the growing membership of the group. It attracted a constant stream of young postdoctoral collaborators some of them working closely with Stephen for several years. Its fields covered neurochemistry and fine structure and later on began to extend to genetics and immunochemistry. It became a unique and leading institution and, due to the teaching and research activities of its members at the 'summer residences' of Woods Hole and the Salk Institute, its

influence extended far beyond the confines of Harvard Medical School.

Stephen Kuffler and his family had settled happily in America, and since 1946 all his research work was accomplished at different laboratories in the United States. He became naturalized in 1954 and undertook various public duties, as a consultant to the Public Health Service and to the U.S. Army, as a Trustee of the Marine Biological Laboratory and as editor of physiological journals. He did also a great deal of travelling overseas, attending conferences, international congresses, honorary degree ceremonies and giving courses of lectures. It started in 1949 when he attended some of the first European postwar symposia in Madrid and Paris; in 1956, on a Guggenheim Fellowship, he took the whole family plus motor car to England and spent several months as a visitor at University College London. Later there were many more trips, to Europe, Japan, India, Australia and South America, but mostly only for quite short periods without ever seriously interrupting the research work at his home base.

The first indication of his illness came in November 1957 when he was being tested medically for some job with the U.S. Civil Service and signs of glaucoma were detected. I have mentioned the succession of medical problems and operations which afflicted Stephen without his letting them stop his work or even his holiday occupations. He continued to play tennis and to go for long swims from his Woods Hole home until the very end, and he still enjoyed travelling abroad. On his last visit to Europe, in the summer of 1980, he had a very busy schedule, receiving an honorary degree at Oxford, going on to Paris and then to Munich to give a Heisenberg lecture, attending the International Physiological Congress in Budapest and visiting Vienna on the return journey to Boston and Woods Hole, where he continued with his latest work.

There had been incidents which caused his family and friends great anxiety: Stephen had adopted a routine of self-administering insulin in the morning and following it with a regular intake of glucose to prevent his becoming hypoglycaemic during the day's work. At times this did not suffice, and there were dangerous episodes when he lapsed into unconsciousness. On one occasion he was discovered by his friends who had been alarmed because he failed to turn up for his lecture. On 11 October 1980, after his accustomed long swim in Buzzards Bay, he felt unusually exhausted and later that day died from a massive heart attack. He was buried at the place he had chosen, in a green field in Woods Hole. A Memorial Meeting was held at Harvard University Memorial Church on 3 April 1981.

Stephen Kuffler was a very fortunate person: he had a very full and happy life, his work was his main hobby and he died in the midst of it. He had received many honours and awards from the scientific community, who treasured him not only for his work, but as a warm-hearted friendly person with quite unquenchable, sometimes even mischievous sense of

humour. He was always ready to demolish pretentious nonsense and to deflate pomposity wherever he detected it. The photograph reproduced as a frontispiece is excellent and shows the almost irrepressible humorous glint in his eye. He liked to poke fun at himself, and he had a special knack of defusing tense situations by making them appear ridiculous before any harm was done. On occasions, he showed a 'chaplinesque' way of dispersing solemnity into a joke: for example, having just been given a flowery introduction to a formal lecture, he pretended to embrace the chairman who was about to attach the microphone round his neck, thus making it appear that he was at a ceremony receiving a most-coveted decoration! At times Stephen Kuffler tended to exasperate his friends, by appearing not to take them seriously and by turning away with a joke or a pun their attempts to raise deeply felt issues; some were critical of what seemed to them an aloofness from political issues and a reluctance to discuss controversial matters. In fact, however, he was a most generous person and very much concerned about others; if he avoided long arguments and disputes, it was because he was conscious that 'life is short', that there was not too much time left for him and most of it he wanted to keep for his top priority, which was his work.

Research work

Australia, 1940–45

Kuffler's first 2 years at the Sydney laboratory were a period of apprenticeship in neuromuscular research, an introduction to the technique of electric recording from cat and frog muscles, to the differences between local depolarizations in the muscle fibre, such as the 'endplate potential' set up in the region of synaptic contact by a nerve impulse, and propagated action potentials ('spikes') which, once initiated at the endplate, travel without fading to the ends of the fibre. He also found himself buffeted by continuing arguments about the role of acetylcholine as chemical mediator in the process of neuromuscular transmission and the somewhat mythical concept of a direct 'detonator' action by the nerve impulse which lingers on in one of the joint papers with J. C. Eccles (4), though its experimental foundations had all but vanished by then.

1. *The isolated nerve-muscle junction*

There is little to be said, from Kuffler's point of view, about the first half dozen joint papers in his list of publications, beyond his own comments that he did not feel very happy about his personal contributions until he took time off, at Eccles's suggestion, to learn how to isolate frog muscle fibres and for the first time to prepare an isolated nerve-muscle junction. With this he succeeded remarkably well in April 1941, and a month later sent off his first independent paper to *Nature*; the

full version was published in the *Journal of Neurophysiology* in 1942 (with a printer's error antedating the receipt by 1 year). This work was a brilliant technical feat, and it immediately and deservedly put him 'on the map'. The paper showed—much more clearly than had been done before—that between the arrival of a motor nerve impulse and the start of a muscle impulse there is an indispensable intermediate step in the form of a local depolarization of the muscle fibre which from previous work was known as the 'endplate potential' (e.p.p.). The experiments left little or no credibility for the 'detonator' concept according to which a muscle impulse could be initiated by the nerve action currents without intermediate local depolarization.

Kuffler used an interesting method of recording which had a number of technical advantages and some flaws: the muscle fibre with its end-plate area was kept in a bath of Ringer's solution, but the region from which he recorded rested on a fine platinum wire embedded in a glass hook and was pushed up against a layer of liquid paraffin. The other recording electrode was a wire in the large Ringer bath. It is clear from Kuffler's interpretation that he did not, at that time, fully appreciate that this method effectively records the potential difference between closely adjacent points of the fibre surface, at the exposed top of the platinum wire and the edges of the glass rod, separated from one another by approximately 0·1 mm. This 'differential' method of recording results in a wave form that approximates to the time course of the local electric *currents* as they enter into, or emerge from, the activated region of the muscle fibre, but they do not correspond directly to the local membrane *potential* change, and some of Kuffler's conclusions, regarding the height of the endplate potential and its supposed equality with the muscle spike, were subsequently found to be mistaken.

During the next 3 years, Kuffler went on to improve and exploit his isolated synaptic preparation and published a series of papers which added quantitative details and clarified previous information on nerve-muscle transmission. He studied the changes of the e.p.p. during the refractory period left behind by a previous nerve or directly initiated muscle impulse. He made experiments on the interaction between the neural transmitter and the muscle spike in normal and partly curarized fibres. He examined the depolarizing action of locally applied acetylcholine and confirmed the large and selective sensitivity to this substance of the junctional region of the muscle fibre. He also measured the increase of acetylcholine sensitivity of chronically denervated muscles, but his method of local application, with a droplet covering 2 mm of fibre length, did not allow him to discriminate between endplate and 'extrajunctional' receptors. The important fact that supersensitivity of denervated muscle is due to a spatial spread of receptors along the fibres rather than a local change at the junctional site was only established later in other laboratories.

He studied the different actions of calcium and calcium deficiency on nerve-muscle transmission and in further experiments produced artificial tetany in frogs and cats by surgical removal of the parathyroid glands. There were several other contributions on the single fibre dealing with the actions of veratrine and re-examining the question of whether the local electric excitability of the endplate differs from the rest of the muscle fibre.

As was to be expected, the use of such a uniquely suitable preparation helped to produce much more clear-cut and convincing evidence than was previously available. But a large part was a confirmation of existing ideas, and there were a number of weak points in this early work of which Kuffler was very conscious later on. Thus, there is an inexplicable erroneous statement in the 1943 paper on the 'specific excitability of the endplate region' (10), namely that local potassium application gave rise to impulses only at the endplate, a conclusion which he later corrected (see paper 17), and there are some doubts also about the quantitative effects ascribed to caffeine in paper 10. In retrospect, one also wonders how, in his experiments on the interaction between directly excited muscle spike and nerve-released transmitter, Kuffler missed the important finding that the e.p.p. can interfere with and substantially reduce the peak of the action potential. This observation which provided important clues to the ionic mechanism of the transmitter action was left to others several years later. One can only guess why Stephen failed to see it; perhaps he did not choose optimal time intervals for this effect, or he worked under conditions of less than optimal acetylcholine release which might have made the change too small to attract his attention.

It has sometimes been claimed that the electrophysiological work on the single neuromuscular junction was decisive in establishing the role of acetylcholine as the transmitter, but this is quite unrealistic and was certainly not the view taken by Stephen Kuffler himself. In a letter to Otto Loewi, dated May 1948, he wrote: 'On the whole my story has not contributed anything new to the well known notion that conduction along the axons is different from transmission across the junction.' The fact is that chemical mediation by acetylcholine at the neuromuscular junction had been established by the experiments of H. H. Dale, W. Feldberg, Marthe Vogt and G. L. Brown in the 1930s. Later on, the application of electrophysiological microtechniques with their special resolving powers for brief localized events was able to throw much light on detailed aspects of the process without fundamentally altering the basic proposition. It is of course true that a novel concept like the acetylcholine story needed many years to sink in, and that all kinds of objections were raised, even after Stephen's work, on premises which did not stand up to further tests. But while the strength of the original theory may have been enhanced in the eyes of those who, following Karl Popper, tried and failed to 'falsify' it, the unsuccessful 'falsifiers' can hardly claim very much credit for it.

2. *Applied electromyography*

There were two other lines of research pursued by Kuffler during his Sydney period: these were human electromyography and its clinical applications, in conjunction with Major A. M. Harvey, and, later, a study of the crustacean muscle system with its antagonistic, excitatory and inhibitory nerve supply, in which I collaborated with him.

Harvey and Kuffler developed a simple method of recording action potentials of human limb muscles in response to electric stimulation of the motor nerve, and using it to assess the extent of lesions and recovery in the peripheral nerve-muscle system (papers 11, 12, 14, 15).

3. *Nerve-muscle transmission in Crustacea*

The work on neuromuscular transmission in Crustacea concentrated on a number of features by which this system differed from that of vertebrates. Apart from the presence of a peripheral inhibitory nerve supply, and the very pronounced facilitation of the muscle response during repetitive stimulation, the most important difference is that contraction of crustacean muscle can be initiated and activated to a large extent via local non-propagated potential changes which are analogous to the e.p.p. At high frequencies of stimulation, full-size action potentials which are capable of propagation can also be elicited in some of the muscle fibres. But in contrast to vertebrate muscles, these spikes are a supplementary and not the principal mechanism for the activation of contraction, intensifying the process without being essential (moreover, crustacean muscle action potentials are now known to be 'calcium' rather than 'sodium' spikes). To a large extent, these experiments supported the views of Wiersma and van Harreveld who had obtained evidence that the activity of crustacean muscle fibres was graded, and not of the all-or-none type encountered in the vertebrates. In particular, neuromuscular facilitation in Crustacea arises mainly from a progressive increase in the amplitude of the local 'e.p.ps' and not from all-or-none recruitment of additional whole muscle fibres.

These experiments also provided a strong indication that depolarization of the muscle fibre membrane is directly related in a quantitative manner to activation of contraction in the interior of the cell. This relation was studied in more detail by Kuffler on isolated muscle fibres of the frog subjected to various electrical and chemical agents which initiate local contractures (paper 19). In every case, activation of the contractile process required depolarization of the fibre surface. When the local potential change exceeded a certain 'threshold' level, the fibre began to contract in that region, and the strength and duration of the mechanical response were graded and varied with the size of the depolarization. A particularly striking observation was that any local contraction, induced by the application of potassium or a depolarizing drug, could be made to relax promptly by electrically repolarizing the fibre surface.

A second paper on crustacean muscle dealt with the response to inhibitory nerve impulses. Marmont and Wiersma had made the interesting observation that, depending on the times of arrival of inhibitory and motor impulses, the former can stop the contraction without interfering with the electrical response of the muscle, that is without reducing the size of the e.p.p. Kuffler and I confirmed this observation, though later work suggests that our method of extracellular recording from the whole muscle was inadequate and failed to reveal a significant shortening of the e.p.ps. This would be quite enough to explain the apparent paradox, for it would mean that the maintained average depolarization of the muscle fibres, during repetitive nerve activity, would decrease substantially. When the inhibitory impulses arrived a few milliseconds *before* the start of each e.p.p., then the amplitude of the latter did become greatly reduced. We wrongly attributed this effect to a 'curare-like' action of the inhibitory transmitter on the postsynaptic receptors. As was shown later by Dudel and Kuffler, it arises from a separate process of 'presynaptic inhibition', that is an interference by the inhibitory transmitter with the activation of the motor nerve endings and their ability to release the normal quantity of excitatory transmitter substance. In short, the 1945 work on synaptic inhibition in crustacean muscle was merely an interesting forerunner of later work done with intracellular recording technique, and its conclusions needed drastic revision.

Chicago, 1945–47

4. *The 'slow muscles' of the frog*

When Stephen arrived in Chicago, R. W. Gerard drew his attention to a paper by Tasaki and Mizutani which suggested that the discarded hypothesis of a separate system of 'tonic' muscle fibres (i.e. non-propagating elements which contract and relax very slowly) may after all have some substance. Since he had recently encountered a somewhat similar system in Crustacea, the idea of pursuing the matter in vertebrates must have appealed to Stephen, and he chose a suitable frog muscle, the *extensor longus* of the fourth toe, for this purpose. In a preliminary paper (22), he reported two types of muscle response to stimulation of a group of small motor axons, one consisting of small local e.p.ps of slow time course associated with barely visible movement, but which summated effectively during repetitive nerve impulses to build up a strong local contraction. The other response was the typical propagated muscle spike accompanied by a fast twitch. In his first paper, Kuffler was evidently thinking in terms of the crustacean analogy; he suggested that both types of response occurred in the same muscle fibre and could be elicited by different rates of stimulation of a single small nerve axon, whereas a large axon would only evoke the propagated response. In the

full paper (24) published a year later, doubts are expressed about this interpretation, and the question of a dual type of response and of innervation in a *single* muscle fibre is left open. The matter was settled several years later, by Kuffler and Vaughan Williams (and reinforced by the work of Burke and Ginsborg), showing unequivocally that two quite separate nerve-muscle fibre systems exist side-by-side, each giving only one type of response: (i) large motor axons supplying propagating twitch fibres, and (ii) multiple small axons connected to non-propagating slow fibres. Moreover, the two types of muscle fibre have different histological and ultrastructural appearance.

The small nerve axons of the 'slow' system have a relatively high threshold when tested with electric stimuli, but the normal reflex activation of their spinal neurons occurs at quite a low level of sensory stimulation and, in fact, slow muscle fibres show a great deal of 'spontaneous' background activity in spinal preparations. It is a system peculiar to the frog and well suited for the maintenance of prolonged slow contractions; the old idea of a separate type of 'tonic' muscle response useful for holding operations and posture was thus resuscitated in quite an unmistakable fashion.

The immediate reaction when the paper was submitted to the *Journal of Neurophysiology* was a letter dated 9 March 1947 from John Fulton, its chief editor, saying

> 'We do not believe that the work is well controlled and we are fearful that you are bringing up once again the old red herring of dual innervation which did so much to retard the progress of neurophysiology after the last war.'!!

Professor J. F. Fulton was a recognized authority in the field of neurophysiology, well known for his textbooks and not prepared to tolerate contradictions to what he considered established physiological principles. He had already been engaged for 10 years in a struggle against the chemical transmission theory, and was not now going to put up with the suggestion that muscle tone could be due to anything other than the classical type of impulses. However, in spite of this rather pompous editorial condemnation, it did not take very long before the conclusions of the paper were accepted by all physiologists; in fact the paper did appear in the same year, and I do not suppose that Stephen had any further trouble from this source.

Baltimore, 1947–59

During his 12 years at the Wilmer Institute of Ophthalmology at Johns Hopkins Medical School, Kuffler made some outstanding contributions to neurophysiology, whose importance was matched by their technical elegance. The highlights were the very influential work on 'concentric'

receptive field arrangements of retinal ganglion cells, done with a superb *in situ* technique in the mammalian retina, and the study—with C. Eyzaguirre—of synaptic inhibition in the crustacean stretch receptor neuron, some of whose records decorate most modern neurophysiological textbooks. There were also the important further experiments, with E. M. Vaughan-Williams, on the slow-fibre system of the frog, and the investigations, with C. C. Hunt, of the intrafusal motor system in the cat.

5. *More on neuromuscular transmitter action*

But before applying himself to these new tasks, Stephen returned for a short time to the old problems of the neuromuscular junction in preparation for a special symposium paper he was to present at the 1948 Federation Meetings at Atlantic City. This paper is of historical, rather than scientific interest, in that it illustrates the perennial antagonism towards the chemical transmission concept which still seemed to be dominant at that time in Stephen's scientific environment. He reported some new experiments in which he showed that there was an irreducible delay between the moment of excitation of the prejunctional nerve twigs and the start of the e.p.p., amounting to about 1 ms in the frog at 20 °C. This was indeed difficult to reconcile with the basic postulate of electric transmission, namely that the currents produced by the nerve impulse could spread directly to the muscle fibre and excite it. It therefore gave indirect support to the acetylcholine hypothesis. However, the main body of the paper is devoted to a long argument about the nature of the agent by which the nerve impulse sets up an e.p.p., and I remember at the time being struck by what seemed to me an attitude of bending over backwards to an extent that must have been painful. In our papers on the effects of curare and eserine in 1941–42, our consensus had been that, without making unreasonable assumptions, all our observations were compatible 'with the hypothesis that acetylcholine is responsible for all the potential changes set up by nerve impulses'. But in the meantime J. C. Eccles had reconsidered the matter, and as late as 1946 he was still fighting a rearguard action against the acetylcholine story at the nerve-muscle junction. Moreover, some of the most prominent neurophysiologists in the United States remained highly sceptical about the chemical transmission ('soup-versus-spark') concept, and clearly did not appreciate the weight of the evidence (or perhaps refused to read the papers) on which it was based. Under these circumstances, it is no wonder that Stephen was extremely cautious in discussing the pros and cons of electrical versus chemical transmission, though even then I felt that his time could have been more usefully employed, and it was rather irritating to see him put the word transmitter in inverted commas whenever he used it, and the final conclusion regarding the nature of the 'transmitter' to read like this: 'It is thought that ions liberated during the "breakdown" in the nerve terminals could best account for the observed phenomena'!

6. *The intrafusal motor system in the cat*

The work in Chicago had shown the existence in the frog of a system of small motor axons which produced a 'tonic' type of contraction unaccompanied by propagated muscle spikes. After that, it became important to find out whether a similar system was also present in mammals. Suggestions of this kind had appeared from time to time, but when Kuffler and Hunt investigated the matter in the cat, they found a very different situation. Confirming and extending earlier observations by B. H. C. Matthews in 1933 and by L. Leksell in 1945, they showed that stimulation of the small diameter axons in the ventral roots of the cat caused no visible muscle contraction, but gave rise to an increased rate of firing of sensory impulses originating in the muscle spindles, presumably via activation of intrafusal muscle fibres. The experiments were done by separating spinal nerve roots into small fibre bundles which made it possible to stimulate single small axons in the ventral root (supplying a particular muscle) and to record impulses from a single dorsal root axon which originate in a spindle of the same muscle. Thus, while there is a clear division between large and small motor axons in the frog as well as the cat, the small axons serve different functions in the two animals. In the frog, the small nerve fibres supply a slowly contracting, 'non-propagating' set of muscle fibres, whereas in the cat they serve to 'pre-stretch' and thereby increase the mechanical sensitivity of the muscular 'strain gauges'. (Incidentally, the frog also possesses an efferent motor system for activating its intrafusal muscle fibres, but it is done in a 'cheap' way through branches of the ordinary motor axons; hence, adjustments of the spindle 'bias' occur merely as an accompaniment of general muscle activity. Unlike the cat, the frog does not possess a separate reflex system for controlling its spindle sensitivity.)

7. *Retinal receptive fields and 'lateral inhibition'*

Stephen Kuffler's incursion into retinal physiology might have been regarded simply as an outcome of his appointment at an ophthalmological institution. There is no doubt that the help and advice given by Dr. S. A. Talbot was instrumental in promoting these experiments. Talbot had designed a multibeam ophthalmoscope which enabled Kuffler to record impulses from single retinal ganglion cells under ophthalmoscopic control of the electrode position and of the stimulating light spots in an otherwise intact cat's eye. Stephen Kuffler was not particularly interested in problems of photo-reception or the sensory cells of the retina. He was using the optic neurons as a suitably accessible outpost of the brain and wanted to study the interaction of excitatory and inhibitory influences on a central nerve cell, further pursuing the problems he had been investigating previously in the crustacean neuromuscular system. His particular object was to examine the limitations and sharpness of the 'receptive field' of the cat retinal ganglion cells, building on earlier

observations by H. K. Hartline. He found that each neuron responded to light spots falling on a discrete circular field of the retina, one or a few millimetres in diameter, but the responses were of opposite kind depending on whether the light impinged on the central area or the peripheral fringe of the field. If in any one neuron, illumination of the centre produced 'excitation' (i.e. an increased rate of impulse discharge), illumination of the peripheral fringe caused a reduction or stoppage of impulse activity (i.e. 'inhibition'). In other neurons these effects were reversed, that is light spots inhibited the neuron if they impinged on the centre of the field, and excited if they fell on the periphery. This type of functional organization in which converging influences from many retinal receptors and intermediate cells interact renders an assembly of optic neurons highly sensitive to the movement of a small object across the visual field, and in particular it will serve to enhance the 'contrast' between adjacent areas of different light intensity. This was pioneering work which must have influenced the research on the functional organization of the visual cortex pursued subsequently in Kuffler's department by his colleagues Hubel and Wiesel. It should be noted that quite independently similar results had been obtained at the same time by H. B. Barlow who was studying the organization of the receptive fields of frog retinal ganglion cells. A few years after this work was done, Barlow joined Kuffler's laboratory, and together with R. Fitzhugh they published a series of papers on various special aspects arising from the earlier study, in particular on the continuous spontaneous firing of optic neurons in complete darkness, and on the changes in visual threshold and the alterations in the size and organization of the receptive fields during dark adaptation. One gets the impression that these subjects were of more immediate interest to his colleagues than to Stephen himself, who by that time had gone on to look for other preparations and for more direct techniques to study the interaction of excitatory and inhibitory synapses on single nerve cells.

8. *The crustacean stretch receptor neuron and synaptic inhibition*

He found a most suitable experimental object in the large sensory neurons in the lobster and crayfish 'tail' muscles. These are stretch receptors, natural strain gauges like the muscle spindles of vertebrates. Their structure and pattern of innervation had been well described by J. S. Alexandrowicz, and their sensory function had been shown by Wiersma and his collaborators. Kuffler was joined in the course of this research by C. Eyzaguirre. Most of the experiments on Crustacea were made in the Woods Hole laboratory where there was a plentiful supply of splendid specimens which were found useful for experimental as well as culinary purposes.

The crustacean stretch receptor neuron with its various sensory and 'modulatory' synaptic connections presents an intriguing miniature

nervous system on its own, and thanks to its large size, the cell body, axon and dendrites and the influence of regulatory impulses in the axons supplying the attached strand of muscle fibres, and in the inhibitory nerve fibre contacting the receptor neuron, all these can be studied directly with micro-recording techniques. The outstanding feature of the series of papers that Kuffler and Eyzaguirre published are the beautiful records which illustrate in a most impressive manner the sensory 'generator potential', that is the local depolarization set up in the dendrites of the receptor neuron by stretch or contractile activation of the attached muscle fibres, and the potentials due to stimulation of the inhibitory axon. These tend to 're-polarize' the neuronal membrane and hold it at or near its resting potential well below the firing level at which sensory impulses are initiated and discharged along the axon process. The quantitative relation between generator potential and impulse frequency, the process of adaptation, the site of origin of propagated impulse activity, the membrane conductance change during inhibition and the 'null-point' (or 'equilibrium level') of the inhibitory potential change, all these were measured and illustrated in a most decorative manner. Kuffler and C. Edwards further studied the action of gamma-aminobutyric acid which at that time was not yet established as the natural inhibitory transmitter, but appeared to reproduce its effects quite faithfully. Their interpretation of the ionic mechanism underlying the inhibitory process had to be revised later when it became clear that an increase of chloride rather than potassium permeability was involved. But altogether, the study on the stretch receptor neuron was one of the brilliant highlights of Kuffler's research work, and it was elegantly summarized in his Harvey Lecture delivered in March 1959.

Boston, 1959–80

9. *Presynaptic inhibition in Crustacea*

After his move to the Harvard Medical School, Kuffler continued to work on the crustacean neuromuscular system. He was joined by Dr Josef Dudel from Heidelberg, and the outcome was a series of important papers in the *Journal of Physiology*, analysing the effects of excitatory and inhibitory nerve impulses on crayfish muscle. The first two papers showed that, in spite of the different transmitter substances, the mechanism of release from the nerve endings was essentially the same as at the vertebrate nerve-muscle junction: spontaneous 'miniature e.p.ps' could be recorded which formed the quantal unit of the impulse-evoked 'junction potential'; the process of neuromuscular facilitation, although much more striking and extensive in crustacea, is due to a recruitment at each junctional site of additional quantal units, just as in vertebrates. However, the third paper by Dudel and Kuffler which dealt with the mechanism of neuromuscular inhibition disclosed something quite new,

namely that the inhibitory nerve impulse, quite apart from its direct postsynaptic effect on the ionic permeability of the muscle fibre, was able to interfere with the power of the motor nerve impulse to release its normal amount of transmitter. In order to reveal the full extent of this 'presynaptic inhibition', the inhibitory impulse had to be timed accurately so as to arrive at the junction just before the excitatory impulse reached the motor nerve terminals. The effect was greatly to reduce the number of quantal packets of transmitter, and temporarily to lower the frequency of 'miniature e.p.ps' recorded in the muscle fibre. This was, in fact, the first unequivocal demonstration of a process of presynaptic inhibition, for which much evidence has been obtained subsequently in the central nervous system of vertebrates, and morphological correlates in the form of 'cascading' or 'series synapses' were soon found in the electron microscope.

10. *Gamma-aminobutyric acid (GABA)—an inhibitory transmitter in Crustacea*

Dudel and Kuffler also showed that all the effects, pre- and postsynaptic, of the inhibitory impulse could be reproduced by local application of GABA, which naturally led to the next series of experiments, in conjunction with E. Kravitz and D. Potter, on the local distribution of this substance in the crustacean nervous system.

To procure adequate material presented a logistic problem: large crates of lobsters arrived in Kuffler's laboratory, and the post-experimental orgies of crustacean meals must have reached saturation point during this period of his research. After some five hundred lobsters had been 'sacrificed' and their nervous system subjected to a long series of fractionations, ten different amino acids were found which produced inhibitory effects on the neuromuscular junction, GABA being by far the most potent among them. The work culminated in a heroic dissection exercise in the course of which single motor and inhibitor axons were isolated from a large number of animals to produce a total length of 'over 5 metres of lobster axon'. The results were well worth the trouble: the inhibitory nerve fibres were found to contain GABA at a concentration of the order of 100 mM, making up some 0.5% of their fresh mass, whereas nothing (meaning less than 1/1000 of this amount) was found in the accompanying motor axons. Although Kuffler and his collaborators expressed themselves very cautiously concerning the physiological significance of their observations, to most of us this result together with previous findings on the actions of GABA was convincing evidence for its identification as the inhibitory transmitter. This conclusion was strengthened by later work, some of it in Kuffler's laboratory, by A. and N. Takeuchi, and by M. Otsuka, L. L. Iversen, Z. W. Hall and E. A. Kravitz.

11. *Electrophysiology of glia cells*

After this surfeit of lobster axons, Stephen relinquished the field of crustacean neurophysiology. He turned instead to some entirely new and original problems, namely the physiology of glia, the 'satellite cells' which surround all neurons and their axon processes. It would be interesting to know what was the immediate cause for Kuffler's departure into this new area of research. He mentions (paper 68) that the work had been planned for over 10 years, but was not given very high priority. One possible indication was an aside in his recently published paper on the GABA content of single axons: 'one should note that the isolated fibres we analysed were still a complex tissue because the dissections left... the Schwann cell layer around the neuron membrane'. He was evidently a little worried by the perennial problem of whether one is correct in attributing determinations of physical and chemical properties to the nerve cells alone, or whether the results might be vitiated by the unavoidable presence of a glia or Schwann cell envelope. Various speculations were current at the time, ranging from those that attributed to the satellite cells a purely mechanical supporting role to those who thought they might have a trophic function or even actively participate in the process of nerve signalling. This last possibility was unequivocally ruled out by the work of Kuffler and his co-authors.

He was joined in these experiments by several colleagues, notably by D. D. Potter and R. K. Orkand, and by John Nicholls with whom he continued to collaborate closely for many years, first in the laboratory and later in preparing an outstandingly good textbook (*From neuron to brain*, 1976). The experiments were done on the central nervous system of the leech; later the optic nerves of frogs and mudpuppies were also studied. Using intracellular microtechniques, Kuffler and his colleagues could demonstrate that the nerve cells in the leech continued to conduct impulses after their investing glia cells had been removed by micro-surgery. They also showed that glia cells, although electrically coupled to one another by low-resistance cytoplasmic pathways, are not linked to the neurons which they surround and do not themselves give action potentials. A very interesting and novel finding was that glia cells not only contain a high internal potassium concentration like nerve cells, but their surface membrane shows a more highly selective potassium permeability and a higher resting potential than the neurones. The glial membrane can therefore be used—like a specific potassium electrode—as a sensitive index for small changes in local potassium concentration which occur during repetitive activity of the adjacent nerve cells. Potassium ions leave the neurons during their action potentials and are reabsorbed only relatively slowly. They tend, therefore, to accumulate in the narrow extracellular clefts between active neurons and surrounding glia cells and cause a progressive depolarization of the latter which can build up to a level of many millivolts. Thus, some of the slow potential changes

recorded during electro-encephalography are probably due to local depolarization of glial syncytia.

The use of the glial membrane as an index of the extracellular potassium concentration enabled Kuffler and his colleagues to answer certain questions concerning ionic concentration gradients existing between the cerebral blood supply, the immediate environment of the central nerve cells and the cerebro-spinal fluid. While the concentrations of potassium in the blood and c.s.f. were known from direct determinations, the concentration around the neurons could now be measured for the first time, by electrical methods, and was found to correspond closely to that in the c.s.f. Another important observation was made when the sodium in the outside bath was replaced by iso-osmotic sucrose: within 10 s the nerve cells became inexcitable which showed that the extracellular sodium concentration had dropped quickly below the level at which an action potential could be elicited. The process was just as rapidly reversed by returning to normal sodium. The glial membrane potential remained constant throughout this procedure, which indicated that the potassium gradient (and by implication the internal ionic composition of the glia cell) had not been disturbed during the drastic extracellular concentration changes.

The work on the electrophysiology of glia cells was a very interesting and original departure, and was ably summarized by Kuffler and Nicholls in a review in *Ergebnisse der Physiologie* (73) and in Kuffler's Ferrier Lecture in 1965 (74). As they point out, the principal problem concerning the roles of satellite cells in the function of the nervous system remained 'on the table', and many tantalizing questions were left open in the absence of 'more precise information about the biochemical properties of various glial cells and about the nature of neuron-glia interaction'.

12. *Synaptic transmission in autonomic ganglia*

After his excursion into the physiology of satellite cells, Kuffler returned to the study of synapses, seeking a preparation that would allow him to make even more direct observations on single neuronal contacts. In his paper with U. J. McMahan (77) he recounts that 'we have searched for a preparation which would survive well in isolation and in which one could directly observe nerve cell bodies, their pre- and postsynaptic axons and surrounding glial or Schwann cells. We especially wanted to see the outlines of synaptic boutons on the surface of living nerve cells, which would enable us to do experiments on neuron-to-neuron synapses that have so far not been possible.' This search led them to the very thin and transparent interatrial septum of the frog's heart with the parasympathetic nerve ganglia embedded in it. By carrying out a preliminary study, using interference optics combined with electron microscopy and a variety of staining methods, they not only familiarized themselves with the fine structure of the septal neurons, but obtained the most magnifi-

cent pictures of synapses on living cells. This was followed by an experimental attack, in collaboration with M. J. Dennis and A. J. Harris, examining synaptic transmission with the usual electrophysiological microtechniques. This work provided a great deal of detailed information on the acetylcholine-mediated postsynaptic potentials, their quantal composition, the spatial distribution of acetylcholine-sensitive sites on the neuron surface, and their spread after surgical denervation. However, the physiological detail that resulted from these experiments must have come as something of an anti-climax after the beautiful structural study preceding it, for it showed little more than that the main features were very similar to what had been found at other synapses such as the neuromuscular junction.

13. *High-resolution studies on the nerve-muscle junction*

No such feeling is aroused by the next series of investigations, done in collaboration with D. Yoshikami, which mark a return of Stephen's personal attention to the neuromuscular synapse. Viewing endplates on the surface of muscles with interference contrast, and 'cleaning' the fibres with collagenase, they examined the localization of acetylcholine-sensitive sites with even finer spatial resolution that had previously been obtained. They found extremely steep gradients of drug sensitivity, falling to a level two orders of magnitude lower than at the actual synaptic contact area, when the tip of the testing micropipette was moved only 2 μm away. An even sharper decline was found in the spatial decrement of cholinesterase activity when this was examined by ionophoretic application of an esterase inhibitor. This work was followed by a paper with H. C. Hartzell and D. Yoshikami in which they showed an interesting interaction occurring during the release of multiple packets of acetylcholine at a single endplate: normally, the effects of the large number of individual packets, which are discharged by a single nerve impulse at hundreds of adjacent sites, are confined to a very small membrane area and do not interact, because the acetylcholine is rapidly hydrolysed near the site of its release and initial action. But when the esterase has been inhibited, the transmitter can diffuse laterally to adjacent sites and, because of the nonlinear, 'co-operative' summation of its fringe effects, interaction now occurs and leads to a very marked prolongation of the endplate potential.

The most important piece of this series is the last paper by Kuffler and Yoshikami in which they describe a technique of quantitatively assaying the amounts of acetylcholine discharged electrically from a micropipette onto an endplate. This was done by injecting acetylcholine ionophoretically with a series of recorded pulses into a measured droplet of Ringer solution and comparing its depolarizing effect on a given endplate with that of similar droplets containing various known acetylcholine concentrations. The aim of the paper was to obtain an improved estimate of the

minimum amount of acetylcholine needed to reproduce a miniature endplate potential, in other words to get a better idea of the acetylcholine-equivalent of the quantal packet delivered by the nerve. The answer was that, with the pipette placed in an optimal position, very close to the most sensitive spots of the endplate, rather less than 10 000 molecules of acetylcholine had to be discharged to produce a response equivalent to a m.e.p.p. This result was of importance in connection with the 'vesicular hypothesis', i.e. that the presynaptic vesicles seen in the electron microscope are the containers of the quantal packet of acetylcholine, and are able to discharge their soluble contents in an all-or-none manner into the synaptic cleft, by a process of membrane fusion and 'exocytosis'. Previous estimates of the number of acetylcholine molecules needed to produce a quantal response had been much higher and made it difficult to believe that such an amount could be accommodated within a 50 nm vesicle. Kuffler and Yoshikami's results removed this objection.

14. *Slow synaptic potentials and a new transmitter substance*

Kuffler's last researches were devoted to a study of slow synaptic effects recorded in a variety of autonomic ganglia of the frog and mudpuppy. It had long been known that the same transmitter, acetylcholine, can produce very different effects at different synapses. The response may be a depolarization leading to an impulse, or an inhibitory change of ion conductance which stabilizes the membrane potential and prevents the initiation of an impulse in the postsynaptic cell. The effects may also differ in their time course, and in the synaptic latency before the response becomes visible; the latter can vary over more than three orders of magnitude. The fast responses at the motor endplate are presumably due to a direct action of the transmitter, and cease when the acetylcholine molecules dissociate from the membrane receptors. Kuffler and his colleagues (87) studied an additional inhibitory action of acetylcholine in a parasympathetic neuron, which had a much slower onset and longer duration. This late response arose well after the acetylcholine had exerted its brief excitatory action on the same neuron, and presumably long after it had diffused away. There were some characteristic pharmacological differences between the brief and slow responses, indicating that the acetylcholine acted on two different kinds of membrane receptors, the so-called 'nicotinic' and 'muscarinic' types. While the fast nicotinic response may well be due to a direct action of the transmitter itself, the muscarinic response seems to occur only at the end of an intermediate sequence of chemical reaction steps.

Finally, in conjunction with Drs Y. N. and L. Y. Jan, Kuffler made the important discovery of a peptide-operated synapse in sympathetic ganglia of the frog. In his last paper, in a publication of a symposium that was held in his honour at Woods Hole in April 1980, he describes 'events in sympathetic ganglia . . . where release of acetylcholine initiates three

different synaptic potentials: (i) a standard fast nicotinic e.p.s.p. (about 30–50 ms duration); (ii) a slow muscarinic e.p.s.p. (30–60 s); (iii) a slow i.p.s.p. (about 2 s). The fourth synaptic signal, the "late slow e.p.s.p.", lasts 5–10 min and is not caused by acetylcholine. We have evidence that a peptide, resembling luteinizing hormone releasing hormone (LHRH), is secreted by specific axons within ganglia where it initiates the late slow e.p.s.ps.' He then recounts the several lines of chemical as well as electrophysiological experiments which led to this conclusion (intraneuronal detection of a peptide of appropriate molecular weight by radioimmunoassay; detection of its calcium-dependent release in high potassium media; disappearance of peptide after denervation; evidence for its production in the neuron and transport along the axon; characteristic postsynaptic effect of direct LHRH application; specific blocking action of impulse-evoked response by a LHRH antagonist). In the summer of 1980, Stephen Kuffler gave a full report on this work to the International Physiological Congress at Budapest; his lecture was regarded as one of the outstanding highlights of this Congress. Immediately afterwards he returned to Woods Hole, and was actively engaged together with Dr T. Sejnowski in experiments designed to elucidate further the mechanism of postsynaptic action of LHRH. He died in the midst of these activities.

Stephen Kuffler had the good fortune to find endless excitement in his life's work. In the course of it he made many important discoveries and brought new light to many areas of neurobiology. His work adds up to a magnificent volume of physiological research; his experimental results were usually accompanied by beautiful illustrations, which added clarity and gave much pleasure to his readers. Technical accomplishment was one of the outstanding features of his research, but he always used his technical skill for a definite purpose, namely to obtain experimental results which were simple and capable of straightforward interpretation, and in this way could give a clear answer to a scientific question. Theory was not his particular line, nor the development of a grand new concept. What he wanted to achieve was to reveal the inherent beauty of the detailed mechanisms whereby nerve cells go about their daily business. In his experimental pursuits he had the gift of making an almost unerring choice of the most suitable living preparation: in this he seems to have emulated one of his scientific heroes, Ramon y Cajal, whom he describes (86) as 'one of the greatest students of the nervous system, selecting samples from a wide range of the animal kingdom with an almost unfailing instinct for the essential'.

I have been greatly helped in the preparation of this memoir by Dr Phyllis Kuffler, Mrs Margaret Wilmot and Professor Felix Mlczoch who gave me important information about Stephen Kuffler's life in Hungary

and Austria, and I am indebted for much valuable assistance to Mrs Marion Kozodoy and Professor Torsten Wiesel. The photograph is by Fabian Bachrach, Boston.

Appendix I

(a) *Honorary degrees*

1959 M.A. Harvard University
1964 M.D. University of Bern, Switzerland
1972 D.Sc. Yale University
1974 D.Sc. Washington University, St. Louis
1974 D.Sc. University of London
1977 D.Sc. University of Chicago
1977 D.Sc. Pierre and Marie Curie University (University of Paris)
1980 D.Sc. University of Oxford

(b) *Membership*

National Academy of Sciences
American Academy of Arts and Sciences
The Royal Society (Foreign Member)
Royal Danish Academy of Sciences and Letters (Foreign Member)
Austrian Academy of Sciences (Foreign Member)
Physiological Society (London)
American Physiological Society
American Philosophical Society
Bavarian Academy of Sciences (Foreign Member)

(c) *Awards and Special Lectureships*

1957, 1980 Bishop Lecturer (Washington University, St Louis)
1959 Harvey Lecturer
1965 Ferrier Lecturer (Royal Society, London)
1971 Silliman Memorial Lecturer (Yale University)
1971 Passano Award
1972 Sherrington Lecturer (Liverpool University)
1972 Louisa Gross Horwitz Prize in Biology
1973 Proctor Award in Ophthalmology
1973 Dickson Prize in Medicine
1976 Wakeman Award
1977 Armin von Tschermak-Seysenegg Prize (Austrian Academy of Sciences)
1978 Gerard Prize
1979 F. O. Schmitt Prize in Neuroscience (M.I.T.)
1979 Forbes Lecturer (Grass Foundation)
1980 Heisenberg Lecturer (Munich)

Appendix II

A curriculum vitae of sorts

By Stephen W. Kuffler

[Written probably in 1971—B.K.]

I spent the first ten years of my life in Hungary on a medium-sized farm. My most vivid memories are about riding horses, swimming in ponds and occasionally visiting a near-by 'big' city. At home we spoke

Hungarian and German and learned reasonably good French from a succession of governesses. A few attempts in elementary education on a private basis by the local school teacher ended in complete failure. This existence came abruptly to an end in 1923 when I was sent to a boarding school near Vienna—a 'gymnasium' run by the Jesuits. Before they let me start on the school's eight-year course they wisely provided a year of preliminary education in order to catch up with my missing elementary schooling. After the preparatory year I stayed on for the full remaining eight years. The education was 'humanistic', with emphasis on moral rectitude of the proper sectarian variety, and eight years of Latin and six years of Greek, and practically no science.

When I finished high school in 1932, I entered medical school in Vienna, after having rejected languages and law, both of which interested me for some undefined reason. I guess I chose medicine for its international character. I spent barely more than five years in medical school, finishing my examinations during the late fall of 1937. It was a difficult and turbulent period in Austrian politics, and we had anything but a peaceful time. While I had many good friends, I strongly rejected my environment—full of violence and social unrest. I spent as little time as possible at the medical school and managed to be away a great deal. There was a period of about six months when, while still a medical student, I worked as an assistant in a hospital in the east end of London. My favourite place was the accident room, where the attending surgeon frequently let me do odd jobs. At the same time I was able to observe the local characters and learn some English. I formed a strong liking for London. Another break in my medical education was a protracted trip of tramping through the Middle East to Egypt and back again.

At the University, I was totally unprepared for the 'scientific' part of my medical courses, particularly in chemistry. This was a handicap that I have never overcome in my subsequent career. Otherwise, my main interest was pathology, which I regarded as the basis of good medicine. I may add that during my medical school years we had a total financial collapse in our family fortunes, and for long periods I made my living as a tutor of retarded high school students, at last making full use of my previous education in Greek and Latin.

On finishing medical school I began a residency in internal medicine and also worked simultaneously in the Department of Pathology in Vienna. This brief clinical exposure was terminated in March 1938, by the German invasion of Austria. Since the next war was close on the horizon and I observed the stepped up brutalities in my environment, I immediately decided to leave, hoping that the conflict would find me on the correct side. I also decided to go to an English-speaking country, preferably England. Shortly after the invasion I escaped by crossing over into Hungary and found my way to London, where I had some good friends. However, I could not use my medical qualifications and decided

to apply for a visa to Australia. During my three months in England, in 1938, I had a transient non-medical job near Manchester, and I spent some time with the family of my good friends John and Charis Brophy. Brophy was a literary figure, one of the sweetest and most generous people I have ever known. We kept in close touch until 1965, when he died, but I still visit his wife Charis each time I come to London.

I bought a ticket to Brisbane, Australia—the last stop on the Orient Line, because it made practically no difference whether one got off at Perth, Adelaide, Melbourne, Sydney or at the last stop Brisbane. Anyway, I left the boat in Sydney and after two days or so of looking around, went to the university and immediately got a job as a demonstrator in pathology. This job lasted for approximately ten days or even two weeks, because in the meantime I met John Eccles who had a tennis court in his backyard, where we had a very good game. On the strength of my tennis he offered me a job as his assistant, which I accepted, not really knowing what it implied. Eccles's enthusiasm convinced me that solving the problems of one's brain, of consciousness, not to speak of such small matters as behaviour, etc. seemed a worth while task for a young man in my situation.

My work with Eccles, however, started on a much more mundane level, exploring the mysteries of the neuromuscular junction. I floundered around terribly and Eccles was extremely patient with me. After a year, in the fall of 1939, we were joined by Bernard Katz from the Department of Biophysics at University College where he had worked with A. V. Hill. We had a very fruitful few years together. The challenge of working with Eccles and Katz was almost unbearable, because both of them were so highly trained and intelligent. Since I could not compete on the intellectual plane, I took full advantage of my manual skills by preparing the first isolated nerve-muscle junction. The simplicity of that preparation compensated for my lack of sophistication, and I obtained my first independent scientific results.

The war soon disrupted the scientific collaboration between Eccles, Katz and myself. Katz and I were keen to enlist. This was easier for Katz who had become a British citizen and therefore could join the air force as a radar officer. My stint in the Australian army lasted only between thirty and sixty minutes. At the induction centre I was issued a uniform and told that I would be sent to the interior of Australia to help in road construction. This was contrary to my understanding of becoming a medical officer, preferably in the overseas forces, and I immediately claimed my status as a doctor. The refusal of the Australians to accept me in the 'proper' army was due to my being a stateless person, since I never accepted German citizenship and Austria had faded away. Anyway, the conflict was resolved by my leaving the induction station.

I had more success with the Americans, who had set up a field hospital in the Sydney area, mainly staffed by people from Johns Hopkins

University Medical School. There I functioned as a consulting neurologist working on nerve injuries that were quite plentiful in the Pacific war. These activities, however, were not full-time and I continued research work in Eccles's laboratory, even after Eccles had left for New Zealand in 1943.

Eccles's laboratory was not reconstituted after the war and Katz and I were looking for jobs. He got one at University College in biophysics, while I obtained a fellowship to go to work in Gerard's laboratory in the United States. By that time I had become a British citizen, acquired an Australian wife and one infant daughter (in 1945). I stayed in Chicago from late 1945 to early 1947, when I was offered a job in the Department of Ophthalmology, the Wilmer Institute, at Johns Hopkins Medical School.

Hopkins was a wonderful place, and they gave me a small basement laboratory that soon became filled with a group of eager young postdoctoral workers, several of them from abroad, particularly Great Britain. By the middle 1950s the place became unbelievably crowded with capable and productive young people. This state of affairs was noticed by Harvard Medical School and they offered us space and opportunities. About ten of us migrated to Massachusetts and eventually we became the founding fathers of a new Department of Neurobiology. Six of the original Hopkins group are still together, but naturally each of us has his own independent laboratory.

In 1954 I became an American citizen and have voted in every major election ever since. Our family of two boys and two girls, now successfully matured, are at the centre of my non-scientific interests. Otherwise my preoccupation remains the nervous system.

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