BIOGRAPHICAL MEMOIRS

Bernard Katz. 26 March 1911 —— 20 April 2003: Elected 1952

Bert Sakmann

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Sir Bernard Katz established the cellular basis of synaptic transmission at the neuromuscular junction, the contact point between nerve and muscle. With his death, we lost one of the most distinguished biophysicists of our time. He laid the foundations for our understanding of almost every aspect of synaptic transmission. Bernard Katz revealed the existence of key molecules and formally described their interaction. With the benefit of his almost magical intuition, he formulated hypotheses that are now recognized as facts.

During his career he pioneered research in three areas. He and Alan Hodgkin elucidated the ionic basis of the action potential overshoot, as formulated in the sodium hypothesis; he unravelled the biophysical mechanisms that generate the endplate potential; and he clarified mechanisms of transmitter release, as detailed in the quantal hypothesis and the vesicle hypothesis. In particular his work on the neuromuscular junction influenced and led several generations of neurophysiologists, and it continues to do so even though research focus has shifted to synapses in the central nervous system.

Bernard Katz (or BK, as he was known to colleagues and students) trained several generations of young investigators who have been inspired by his hypotheses, by his impeccable thoroughness as an investigator, and by the straightforward, unpretentious style of his presentations—though some have been dismayed by his occasional unapproachability or his unforgiving nature when confronted with others' mistakes! Perhaps his most valuable and enduring legacy to collaborators and students is that, when data are difficult to interpret and we see only a faint glimmer of light at the end of a long tunnel, we can ask ourselves, 'What would BK do now?'

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Family background

BK was born in Leipzig on 26 March 1911, the only child of Morduch (Max) and Eugenie Nahum. Eugenie Nahum Katz (*née* Rabinowitz) (1889–1983) was born in Warsaw to a Polish mother (*née* Glass) and a German father, Bernard Rabinowitz. Max Katz (1882–1971) was born in Moghilev, a town on the River Dnjepr in Belarus, Russia. He was one of the 15 children from the second marriage of Russian fur trader David Katz. Max Katz, like his father, made his living in the fur trade. Because Russian anti-Semitism was particularly virulent, at the turn of the century Max Katz emigrated from Moghilev to Leipzig in Germany some time between 1904 and 1906. In the early 1900s Leipzig was a global centre of fur trading, and Max established a fur shop; in 1909 he married Eugenie in Vienna. After the Russian revolution the family did not apply for Soviet citizenship, so they effectively lost their Russian citizenship. As a result BK and his parents had a stateless persons' pass ('Certificat Nansen') until their naturalization in Australia during the early 1940s.

In Leipzig the Katz family lived on the second floor of a house at 13 König-Johann-Strasse. On the ground floor of the Katz house there was a bakery—perhaps the source of BK's life-long 'sweet tooth'. In the early 1900s, this well-to-do quarter of Leipzig had a relatively large Jewish population, well documented by the Israeli writer S. Y. Agnon in his novel *In Mister Lublin's shop.* Agnon lived in the same street as the Katz family from 1918 to 1924.

From 1917 to 1921 BK attended a local elementary school, and in 1921 he took the entrance examination for the Schiller-Real-Gymnasium and received top grades, but he was not admitted because of racial prejudice. Instead, for the next eight years he attended the König-Albert-Gymnasium for boys (the 'Albertinum'). Despite his early setback he enjoyed his childhood in Leipzig and liked his school (although he was traumatized when another pupil's father reportedly suggested that Jews should be lured into the fur trade hall to be poisoned). He was always top of the class, and the last entry in the class yearbook praised his intellectual abilities, his modesty and his politeness. Because BK followed a linguistic rather than a mathematical course of study he had time for 'playing chess in the cafés of Leipzig'. However, these extracurricular pursuits did not compromise his studies: BK skipped one year of school and passed his final examinations in 1929.

On leaving the Albertinum, BK entertained the idea of studying philosophy and literature. However, with anti-Semitism on the rise he considered emigrating to Palestine. These plans were shelved in favour of studying medicine at Leipzig University. Medicine promised a better chance of earning a living outside Germany and of supporting his parents in their retirement. At the start of his studies he was still thinking of emigrating, so he joined the Zionist student union 'HaTikva'.

Leipzig University (1929–34)

BK began his medical studies in April 1929 and passed his preclinical exam in July 1931. He learnt anatomy and histology from H. Held, who advocated the syncytium hypothesis of the nervous system as opposed to Cajal's neurone doctrine. BK was taught physiology by M. Gildemeister and physics by P. Debye (ForMemRS 1933). BK wrote (in his Society for Neuroscience memoirs) that he felt comfortable at only two places in the university: in Sigerist's Institute for the History of Medicine, and in Gildemeister's Institute for Physiology.

Gildemeister was working on the problem of local excitation of myelinated nerve fibres. BK was attracted to the quantitative treatment of biological phenomena and so joined Gildemeister's institute. In 1933 he won the Garten Prize but was not granted the award for 'racial' reasons. Gildemeister nevertheless presented the prize to BK privately. BK was the president of the 'HaTikva' at the time when he was forced to resign from the students' union in 1933 by the university officials. Because of the increasing influence of the Nazi regime on academic staff, again he planned to emigrate to Palestine. Among the personal acquaintances who remained loyal to him in these difficult times was R. Bachmann, who held the chair of anatomy at Munich University after the war. BK completed his state exams in medicine in November 1934 and wrote his MD thesis on his work in Gildemeister's institute, which was concerned with the seasonal variation of frog muscle membrane permeability, as measured with an alternating-current Wheatstone bridge. His first two scientific articles appeared in *Pflügers Archiv*.

While considering his future outside Germany, BK met Chaim Weizmann in Karlsbad in August 1934. Weizmann (who was to become the first president of Israel) offered to help when he found out that BK wanted to work with A. V. Hill FRS in London. BK had the greatest admiration for Hill, who later became his friend and protector. Hill did everything he could to help people in Germany threatened by the increasing pressure from the Nazi government. Supported by letters from Gildemeister, Weizmann lobbied Hill on BK's behalf and succeeded in securing BK a position in Hill's laboratory. Before he left Leipzig for England in February 1935, BK undertook a brief period of training in internal medicine and surgery at the Eitingon Jewish Hospital, and worked there from the end of 1934 until February 1935.

EMIGRATION (1935) AND EARLY WORK IN ENGLAND ON EXCITABILITY (1935–39)

BK felt 'reborn' upon arrival in Harwich, despite being penniless and having a scant knowledge of English. At A. V. Hill's unit at the Department of Physiology at University College London (UCL) he was initially supported by a Refugees Fund, established by the International Student Service for 'a 2-year PhD course'.

In Hill's biophysics unit (1935–39), BK experimentally tested the predictions of Hill's theory of electrical membrane excitation, specifically those on the subthreshold excitability of myelinated frog sciatic nerve (1)*. BK found that, at high stimulation frequencies, the maximum of excitation is followed by a decrease that he attributed to an electrotonic block outrunning the nerve impulse; that is, a refractory phase (1). In addition, the threshold was lower than predicted, an effect later attributed to a local subthreshold response. Using Hill's theory, he also studied the accommodation of myelinated frog nerves to constant-current electrical stimulation under a variety of conditions, using the duration of the repetitive action potentials (APs) in nerve or muscle as an index of response. He calculated the duration of the period in which the local potential remains above threshold and initiates APs. The calculated value was in accordance with predictions derived from theory (3–5). Finally, the time course of the local electrical response of nerves to subliminal stimulation when using brief double shocks (6) provided further experimental support for the suggestion that a local response preceded an AP.

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* Numbers in this form refer to the bibliography at the end of the text.

These were remarkable results, given that muscle responses were used as an index of excitability. The local response in a nerve was measured more directly shortly afterwards in the much larger crab nerve by A. (later Sir Alan) Hodgkin (FRS 1948). In 1938 BK, testing the theory by using alternating current stimulation (8), found that most of the divergence of experimental results from Hill's theory was attributable to a sort of local action potential of the nerve at higher stimulation strength.

BK became interested in neuromuscular transmission and he therefore used crab nerve fibres, which are larger than those of a frog's sciatic nerve. The work that began in the summer of 1935 in Plymouth continued through the winter in London; it used electrical nerve stimulation and measured muscle contraction (2). BK investigated the 'central properties' such as facilitation and inhibition, and also the blocking effect of a high concentration of extracellular magnesium on synaptic transmission. In his first article on neuromuscular transmission in the frog sartorius-nerve–muscle preparation (7) he showed that in a partly curarized muscle, neuromuscular block can be overcome by a brief catelectrotonus; that is, by polarizing the membrane potential electrically with an extracellular electrode. This was the first indication of a depolarization of the muscle membrane, which later turned out to be the endplate potential (EPP).

During his time at UCL, BK lived with Hill in Highgate, becoming part of Hill's family. This period was the 'happiest but most impecunious time of his life'. He worked mostly on nerve excitability to elucidate the local response of nerve and muscle membranes. In the summer of 1935 BK visited the Plymouth Marine Biological Laboratory for the first time to work on the crab neuromuscular junction, his first foray into synaptic transmission. In 1937 he compiled a review on electrical excitability for *Ergebnisse der Physiologie*, which was originally supposed to be authored by Hill. When the editor and the publisher asked that an Aryan co-author be included, BK demanded the return of the manuscript. A short article appearing in the *Sydney Morning Herald* in 1940 (by BK under the pseudonym Iatros) commented that Mr Winston Churchill might allow his name to be used, but that this would not be appropriate. The review was eventually published as a monograph by Oxford University Press in 1939. BK completed his PhD thesis, 'Excitation and transmission in nerve and neuromuscular junction', in Hill's biophysics unit, and in 1938 he was awarded a PhD from London University.

In 1935, BK met John (later Sir John) Eccles (FRS 1941) at a Physiological Society meeting in Cambridge; Eccles at that time was defending the electrical ('detonator') hypothesis of neuromuscular transmission against the 'chemical transmission' model proposed by Dale and colleagues. BK was impressed by the intensity of the arguments, and also by the friendly personal relations between the protagonists when the meeting was over. Eccles was working at Oxford with Sir Charles Sherrington FRS, but when Sherrington retired from the chair of physiology in 1936 the direction of research shifted away from Eccles's interests. So Eccles, aged 34 years, returned to his native Australia in August 1937 to take up the directorship of a department in the Kanematsu Memorial Institute for Pathology. He soon invited BK to join him there. BK accepted this offer and was awarded a Beit Memorial Fellowship and additional funds from the Carnegie Institution shortly before his departure.

MOVING TO AUSTRALIA (1939) AND PROVING THE ACETYLCHOLINE HYPOTHESIS
OF NEUROMUSCULAR TRANSMISSION

In the summer of 1939, just before the outbreak of World War II, BK and his parents (whom he had managed to escort to England from Nazi Germany earlier that year) boarded a boat bound for Sydney. When war finally broke out in August, they were delayed for a few weeks in Colombo, Ceylon. About 50–60 people in addition to the Katz family had to wait for the next ship, and these people insisted on consulting BK about their minor ailments despite his protesting that he was not a practising doctor. The trio finally arrived in Sydney in October 1939.

Eccles created a research department on the fourth floor of the Kanematsu, overlooking Sydney Harbour. At the end of 1938 Eccles had been joined by S. W. Kuffler (ForMemRS 1971), then aged 25 years. Kuffler, originally from Hungary, had trained in medicine and pathology at the University of Vienna. Eccles, BK and Kuffler together proceeded to lay the foundations of synaptic physiology. The famous photograph of the three of them in Sydney was taken at Martin Place in 1941; the three were probably making their way from the institute to the university. Kuffler and BK maintained a lifelong friendship. BK was, in his own words, a 'frogman', whereas Eccles and Kuffler were 'catmen', initially working on the cat soleus muscle *in vivo*. BK preferred to work *in vitro* on the sartorius muscle of the Australian treefrog, an attractive green and golden bell frog.

In the mid-1930s, the acetylcholine (ACh) hypothesis of chemical transmission at the neuromuscular junction was put forward by Sir Henry Dale FRS, G. L. (later Sir Lindor) Brown (FRS 1946) and W. Feldberg (FRS 1947), whereas Eccles and Sherrington still favoured an electrical mechanism. The endplate potential (EPP) in frog muscle was described in 1938 by Schaefer and in 1939 by Eccles and O'Connor in cat muscle. It was interpreted by both groups (incorrectly) as being due to electrical excitation of the muscle by the nerve terminal, rather than to the action of ACh. The release of ACh during synaptic transmission was known from the work of O. Loewi (ForMemRS 1954) and of Dale, Brown and Feldberg in the 1930s. But it was the work of BK, Kuffler and Eccles which showed that the endplate potential is generated by the action of ACh (9–11) and not by direct electrical stimulation of the muscle via the nerve terminal. This conclusion was based on the effects of the ACh esterase inhibitor eserine on neuromuscular transmission (11). The view of an ACh-induced conductance increase was corroborated by BK's extracellular impedance measurements of the muscle during neuromuscular transmission, showing a very brief (millisecond) action time of ACh (10). Thus, BK and Kuffler eventually convinced Eccles that the 'detonator' theory of synaptic transmission was incorrect.

WAR SERVICE (1941–44)

BK's application for entry into the Royal Australian Air Force (RAAF) in 1940 was not initially accepted because he was a 'stateless foreigner'. He was naturalized in 1941. After the Japanese attack on Pearl Harbor in 1941, the invasion of Malaya, and the fall of Singapore in January 1942, BK offered his services again and was glad to be accepted. His first military posting (October 1942 to March 1943) was with a radar unit on Goodenough Island, situated off the northeastern coast of New Guinea. As a radar pilot officer, and later as a flight

lieutenant, he was in charge of about 20 men running a movable radar unit. The unit surveyed the Japanese planes that were attacking Port Moresby and Darwin, Australia, from their base at Rabaul on the island of New Britain. His second posting (mid-1943 to autumn 1945) was at the Radio-Physics Laboratory at Sydney University, where he was involved in the development of the radar transponder. When in Sydney he worked part time at the Kanematsu with Kuffler, as Eccles had taken a post as head of physiology at University of Otago, New Zealand. In mid-1945 BK was offered the position of senior lecturer in the physiology department of E. Wright at Melbourne University. BK was considering this offer when, in October 1945, Hill offered him a position back at UCL.

Marriage to Rita Penly and moving back to UCL in 1946

During his time at the Radio-Physics Laboratory at Sydney University, BK met his future wife, Marguerite Penly, on a Cremorne tennis court. Rita's love of both BK and tennis was to endure. Born in 1921, Rita worked for the Australian Broadcasting Corporation, overseeing a children's programme. The couple married in October 1945, an event that BK described as 'the biggest personal achievement of my time in Australia'. (A photograph of Rita as a young lady always had pride of place in BK's office.) He and Rita took a passage on the *Moolton* and arrived in London in February 1946.

In 1947 and 1948, BK and Rita occupied the top floor of Hill's house. From 1948 onwards they lived in Kenton Gardens, which was on the same Underground line as UCL. Their marriage was a very happy one, during which they raised two children. Their first son, David, was born in 1947; he is now a doctor practising in Wales. Their second son, Jonathan, was born three years later, and is now teaching classics at Westminster School. In 1960, BK's parents returned to live in London.

Sodium hypothesis of the AP overshoot

By the late spring of 1946, Hill had re-established the laboratory in Gower Street with the help of the Rockefeller Foundation and the Medical Research Council. Positions were offered to a small number of researchers and technical staff, and BK became a Henry Head Research Fellow of the Royal Society. Reflecting the focus of BK's research, the laboratory was known as the Biophysics Research Unit. BK worked on crab nerve excitability and the muscle spindle. Towards the end of 1946, he triggered a collaboration by sending Hodgkin a manuscript showing that crab axons cannot be excited in salt-free solutions. These data reflected Hodgkin's own experience and prompted Hodgkin to test the effect of sodium deficiency on crab nerve fibres by using extracellular recordings. He got an encouraging result in 1947 and decided to expand these studies but in squid axon with BK, the same preparation with which A. Hodgkin and A. (later Sir Andrew) Huxley (FRS 1955) had first recorded the AP overshoot. Hodgkin and BK (14) demonstrated the contribution of sodium ions to the AP overshoot in the squid giant axon. The results were a first indication that in the conduction of an impulse along a nerve, the permeability of the membrane to sodium and potassium ions increases transiently: an increase in the permeability for sodium causes a reversal of the membrane potential from negative to positive. The 'idea' of the sodium dependence of the AP overshoot had arisen

independently through BK's collaboration with Kuffler in Sydney, but now experimental evidence was accumulating. In the following summer Hodgkin and BK were joined by Huxley and used the voltage clamp method that Hodgkin had adapted, to establish the basis of the ionic dependence of the resting membrane potential.

When Paul Fatt (FRS 1969) arrived in London that same summer, he was advised by Hill to contact BK in Plymouth; together they began to work on neuromuscular junction, both in Plymouth and at UCL.

Muscle spindle excitation

BK also studied the mechanoelectrical transducer properties of the muscle spindle, perhaps motivated by the observation of Kuffler that stimulation of small-diameter frog motor-nerve fibres produced a slow local potential change in muscle fibres that is wholly different from the propagated muscle spike recorded when stimulating the axons of larger diameter. He showed with J. Z. Young FRS (15) that, at least in frog, these fibres form an accessory motor innervation system that innervates both the intrafusal and extrafusal muscle fibres. Notably, the intrafusal motor junctions that pull on the muscle spindles are more resistant to curare.

In 1950 BK worked on measuring depolarization in the spindle and AP initiation in the afferent fibres induced by muscle stretch. The spindle potential was measured in the presence of a moderate concentration of local anaesthetic to block the conduction of action potentials (16, 17). In the appendix of the latter article an attempt was made to derive a physical model of the effect of stretch on spindle membrane potential, by analogy with a mechanoelectrical device such as a piezotransducer. BK dismissed the generation of the spindle potential by changes in membrane thickness because the effect predicted by this model was smaller than the experiments revealed. Again, his intuition about a change in conductance was right—the potential was determined by stretch-sensitive ion channels.

Inhibition in crabs

BK's interest in neuromuscular transmission in crab muscle continued after his move to Australia. With Kuffler, BK used extracellular recordings to describe neuromuscular inhibition (12) and synaptic excitation (13). Later, in the Biophysics Department at UCL, BK and Fatt used intracellular microelectrodes to study the interplay between excitation and inhibition (21). The recordings showed that inhibitory transmitter increased membrane conductance, suggesting that inhibitory and excitatory transmitters compete for the same receptor. This view of the interaction between excitatory and inhibitory nerves of the crab neuromuscular junction was clearly an oversimplification, and they failed to draw the correct conclusions from their very meticulous experiments. Dudel and Kuffler later showed that the two transmitter types are liberated independently, and that the interaction between excitatory and inhibitory nerves is based on presynaptic inhibition of excitatory nerve terminals.

DIRECTORSHIP OF THE DEPARTMENT OF BIOPHYSICS AT UCL (1952–78)

When Hill retired in 1952, three candidates were considered as his successors: Hodgkin, Huxley and BK. Because Hodgkin wanted to stay in Cambridge and Huxley was considered by the UCL board to be too young, BK was offered the job. However, he had also been

approached by the Australian National University in Canberra. Preferring to remain at UCL, he requested that the board match the Australian offer. The biophysics unit was thus granted full departmental status with its own staff, with BK at its helm.

The 1950s and 1960s: shedding light on transmitter release by intracellular recording from frog muscle fibres

The experiments in Sydney on the endplate potential provided conclusive evidence for chemical transmission. BK's major subsequent contributions were in elucidating (i) the mechanisms underlying the calcium-driven release of ACh in multimolecular packets, and (ii) the mechanisms underlying the actions of ACh in increasing the ion permeability of the endplate membrane. Rapid progress in understanding these mechanisms at the neuromuscular junction was made possible through the invention of fine-tipped glass microelectrodes, which allowed intracellular recording from single muscle fibres. Previously, BK had observed small electrical 'blips' when recording extracellularly from a muscle. The breakthrough in elucidating the nature of these spontaneous release events (or 'miniature' endplate potentials (MEPPs)) resulted from the use of intracellular microelectrodes (MEPPs have amplitudes of up to several millivolts when recorded intracellularly but are usually only a few hundred microvolts when recorded extracellularly). The key observations were made in the spring of 1950 (18) and were reported in detail in 1952 (2). When Fatt left for Australia to work with Eccles, BK collaborated with J. del Castillo to calculate the statistics of quantal release in the presence of low concentrations of extracellular calcium and high levels of magnesium (22) to develop the quantal hypothesis of transmitter release. The quantal hypothesis refers to the composite nature of the endplate potential. Del Castillo and Katz stated:

> Transmission at a nerve–muscle junction takes place in all-or-none 'quanta' whose sizes are indicated by the spontaneously occurring miniature discharges. The number of quantal units responding to a nerve impulse fluctuates in a random manner and can be predicted only in statistical terms. The average 'quantum content' of the e.p.p. depends on the probability of response of the individual unit, and this varies with external Ca and Mg concentration.

Vesicle hypothesis (1955–60)

At the time of its first formulation, the main evidence for the vesicle hypothesis was purely circumstantial. Del Castillo and BK first alluded to it in 1955 (26) when they suggested—on the basis of the finding that sodium is not required for MEPPs recorded in an extracellular solution with a high concentration of potassium—that the vesicles observed in the electron microscope by Robertson might coalesce with the axon membrane before releasing their contents. They wrote:

> It is possible to imagine a mechanism by which each particle loses its charge of ACh ions in an all-or-none manner when it collides with, or penetrates, the membrane of the nerve terminal.

The first schematic drawings of vesicles in a presynaptic nerve terminal, closed or coalesced, were published in French, as contributions to a CNRS (Centre National de la Recherche Scientifique) meeting in 1957 (29). Later, BK and colleagues did their own electron microscopic study (30). They reported that vesicular structures accumulate at specialized sites of the presynaptic terminal, stating:

> It has therefore been suggested that ACh may be contained in the vesicles and released in an all-or-none manner when the vesicle collides with certain spots of the terminal membrane. Such an event may involve coalescence of the membranes, of vesicle and axon, and diffusion of the vesicular contents into synaptic space.

The anatomical basis of quantal release remained central to BK's thinking, and he inspired J. Heuser, an American postdoctoral worker trained in electron microscopy, to spend a decade working on this question. Heuser joined the Biophysics Department in the early 1970s; in London, and later in the USA, he obtained snapshots of vesicles coalescing with the axon membrane; with T. Reese and colleagues he established the 'vesicle cycle', in which vesicles fuse with and transitorily become part of the axon membrane.

Establishment of the calcium hypothesis with recordings from nerve endings (1954–70)

However, anatomical evidence for coalescing vesicles was not strong at the time. The clarification of the effect of calcium on evoked transmitter release was one of the fundamental achievements of BK and his co-workers. It culminated as the 'calcium hypothesis', which states that 'transmitter release is brought about by an influx of external calcium through special membrane channels which are opened by the depolarizing pulse' (37).

This statement marked the culmination of a 25-year-long effort by BK, and his colleagues P. Fatt, J. del Castillo, R. Miledi (FRS 1970), F. Dodge and R. Rahamimoff, to understand the mechanism of calcium-dependent phasic release of transmitter from nerve terminals in quantitative terms. It was also the preliminary endpoint of a field of research that began in 1894 with Locke's discovery of the calcium dependence of neuromuscular excitability.

Kuffler and BK noted in Sydney that a fivefold increase in the concentration of extracellular calcium increased the amplitude of the EPP (10), and Feng and co-workers in China had suggested that extracellular calcium affects the amount of transmitter released. The observation that calcium increased EPP magnitude was quantified finally in 1952 by del Castillo and L. Stark at UCL, who showed that calcium affected the release of ACh and not its interaction with the endplate. These early results were extended by intracellular recordings by Fatt and BK (20). When investigating the properties of spontaneous miniature potentials, they changed the sodium and calcium concentrations. Decreasing the concentration of calcium reduced the amplitude of the evoked endplate potential in a stepwise manner, whereas the size of MEPPs was not affected. They wrote that 'this effect [of calcium deficiency] appears to take place in steps, involving an all-or-none blockade of a variable number of miniature components'.

Del Castillo and BK then studied the effect of magnesium on EPPs and MEPPs and proposed several mechanisms for the release of ACh, including an 'ACh carrier' molecule. Despite being similar for both spontaneous and evoked release, it was only the ACh carriers mediating the EPP that were active during evoked release. Only the evoked release was dependent on calcium (23).

They also investigated the possibility that arrival of the nerve impulse increases the frequency of MEPPs by polarizing the terminal (23). They concluded that the release mechanism comprised two separate pathways for spontaneous and evoked ACh release. The one that they had favoured in their earlier article was, in fact, consistent with data from their polarization experiments. This insight paved the way for the formulation of the 'calcium sensor hypothesis' in 1954. According to this hypothesis, the step that is common to both spontaneous and evoked release is the liberation of an active carrier molecule ('X') that transports, or allows the passage of, a large number of ACh ions from the presynaptic terminal, leading to the generation of a MEPP (23).

Location of the calcium-binding site

When first formulated, the calcium hypothesis did not specify the location of the calcium sensor, although it implied that the carrier molecule transported the unit of transmitter across the presynaptic membrane. BK and Miledi studied the synaptic delay (31) and concluded that this delay arose primarily from the delayed release of quanta, as a result of a cascade of metabolic intermediates. However, the site of action of calcium ions remained unknown.

Experiments performed by BK and Miledi during 1967–70 established that either Ca or Ca–X had to pass from the extracellular side into the cytoplasm during an AP. This was first concluded indirectly for the neuromuscular junction (34) and then, more directly, for the squid giant synapse (33).

Between 1965 and 1969 BK spent relatively short summer periods at the Stazione Zoologica in Naples, Italy. From its inception, the Stazione was a truly international institution—the Royal Society generously helped to re-establish its laboratories after World War II. In 1965 there were not enough fresh squid in Plymouth to sustain research, but in Naples, where Young had discovered the giant squid axon, there were plenty. A. Packard, the director of the Stazione's zoology department, and his successor, R. Martin, had an arrangement with local fishermen: every day from as early as July there was a fishing boat dedicated to providing Miledi and BK with fresh hand-caught squid.

The Naples squid experiments showed unequivocally that the entry of Ca or Ca–X into the presynaptic terminal was required for transmitter release through the cascade of events

$$\text{depolarization} \rightarrow \text{calcium influx} \rightarrow \text{quantal transmitter release.}$$

At that time, Dodge and Rahamimoff first described the correct stoichiometry of extracellular calcium ion concentration and activation of the putative calcium sensor. They interpreted their results in terms of the formation of a Ca–X complex on the nerve terminal. However, the route for calcium influx was still unclear. It had not yet been proven that calcium ions enter the terminal via calcium-specific, voltage-gated ion channels opened during an AP. Nevertheless, two sets of experiments at the squid giant synapse had made these possibilities highly probable. BK and Miledi showed that a regenerative response of the nerve terminal of the squid giant synapse depended on an extracellular calcium-dependent regenerative potential, and that evoked release was prevented at the calculated calcium equilibrium potential (36). Furthermore, the change in the presynaptic membrane's permeability to calcium was closely related to the increase in transmitter release (37). BK and Miledi concluded: 'Transmitter release is brought about by an influx of external calcium through special membrane channels which are opened by the depolarizing pulse.'

BK also pioneered the study of neuromuscular facilitation. With Kuffler and Eccles he had observed neuromuscular facilitation during extracellular recordings from frog and cat muscle (9). In discussing the calcium hypothesis of transmitter release, BK and Miledi wrote (32):

> The exact place of calcium action in the process of transmitter release remains unknown. … The speed and reversibility makes one wonder whether a phenomenon like facilitation may be due to a residual change in ionized calcium concentration at some important site of the membrane, but it is difficult at present to think of decisive tests for this idea.

However, they cautioned that the result of dual-pulse experiments (35) does not prove the hypothesis that the amount of calcium entering the axon membrane during stimulus N2 adds to the remainder of active calcium left by stimulus N1.

Rahamimoff, then a postdoctoral fellow of BK's, also reported evidence for the residual calcium hypothesis from dual-pulse experiments at different extracellular calcium concentrations.

A Nobel Prize for BK

In 1970 BK was awarded the Nobel Prize in Physiology or Medicine, together with Julius Axelrod (ForMemRS 1979) from the USA and Ulf von Euler (ForMemRS 1973) from Sweden, 'for their discoveries concerning the humoral transmitters in the nerve terminals and the mechanisms for their storage, release and inactivation'. BK was generally not very fond of ceremonies and often used to refer to such an occasion in an ironic manner characteristic of him.

ACh action at the molecular level (1972)

BK made equally fundamental contributions to our understanding of the postsynaptic action of ACh; in 1942 he began to formulate hypotheses that have since become fact. The first electrophysiological evidence that ACh generates the endplate potential (11) came from extracellular recordings from frog sartorius muscle.

By making plausible assumptions it is shown that the observed actions of curare and eserine are reconcilable with the hypothesis that ACh is responsible for all the local potential changes set up by nerve impulses.

This study also showed that the action of ACh is very brief, lasting only a few milliseconds. Evidence for this short-lived effect came from using A. V. Hill's 'local potential theory' to analyse the rise and decay times of the EPP (9), from using the Cole and Curtis transverse impedance method to demonstrate a short increase in conductance at the endplate during transmitter action (10), and finally by showing the restriction of transmitter activity to the muscles endplate region.

As a result of his strong background in the mechanisms of membrane excitability, BK was well aware of different possible ion selectivity mechanisms that could generate the endplate potential, such as complete breakdown of the membrane, cation-selective channels, and separate sodium-selective and potassium-selective increases in permeability. In 1951 Fatt and BK suggested that ACh might short-circuit the membrane (19) by causing a non-specific increase in membrane permeability. In 1954 del Castillo and BK (24) suggested two alternatives for the 'short-circuit' hypothesis: a selective increase in permeability for several ion species and a complete breakdown of a local ion barrier. They also mentioned that there might be specific 'chemoreceptors' (implying ACh receptors) for the EPP and separate specific sodium 'carriers' that mediate the AP.

They later found (26) that MEPPs are present even in the absence of extracellular sodium ions, suggesting that potassium ions are similarly transported across the endplate membrane. The issue of membrane breakdown versus increased permeability for several ion species remained unresolved. Interestingly, they mentioned the first possibility of ACh-operated membrane channels (26). The issue was finally solved when A. and N. Takeuchi used a voltage clamp of endplate currents and ion substitution to show that ACh opens ion channels that have comparable permeabilities for potassium and sodium ions.

Using iontophoresis to map ACh sensitivity, del Castillo and Katz identified localized 'chemoreceptors' for ACh at the endplate (25). They proposed the first realistic kinetic scheme. Here the interaction between ACh and the chemoreceptors, in terms of a modified

version of the Michaelis–Menten equation (28), was described such that ACh could bind to an inactive form of the receptor without causing depolarization and in a second step could transform into a depolarizing receptor complex.

The binding–isomerization scheme for ACh receptor was extended by work with S. Thesleff using W. L. Nastuk's iontophoretic method to apply short pulses of ACh to small, localized areas of the endplate (27). This kinetic approach led to the idea that

> the receptor can exist in two forms, 'receptive' and 'refractory' (i.e. desensitized). The drug combines rapidly and reversibly with both forms but the combined receptor is transformed irreversibly from A to B … .

This reversible cycle requires two states of unliganded receptors with two different affinities, as in the Monod–Wyman–Changeux cyclic scheme.

Elementary events (1970)

The derivation of the properties of molecular events activated by ACh was achieved by Katz and Miledi in 1970 (38). They analysed the additional electrical membrane noise accompanying ACh-induced depolarization, writing: 'We suggest that the membrane noise observed in these experiments arises from statistical variation of high frequency collisions between ACh molecules and end-plate receptors.'

In 1971, with the observation of ACh-evoked membrane noise during extracellular noise recordings made simultaneously with focally recorded endplate currents (39), the elementary conductance changes that generated an endplate potential were estimated. The average duration of an elementary event was 1 ms (at 20 °C). Their elementary conductance estimate of 100 pS was about five orders of magnitude greater than that derived by M. Kasai and J.-P. Changeux from tracer flux experiments on electric organ membrane fragments. Noise analysis eventually led BK and Miledi to propose two alternatives for the time course of the single-channel current: a sudden on-event followed by relaxation, or an abrupt on–off event (40). Direct recordings of currents through single channels made by E. Neher and myself in 1976 on frog muscle gave conductance values that were smaller (about one-quarter), and proved the abrupt on–off event.

RETIREMENT AND EMERITUS PROFESSORSHIP

BK retired from UCL in 1978, at the age of 67 years, having established the Biophysics Department as a world centre for physiology and biophysics. He was succeeded by R. Miledi, who, however, left for California in 1983. The Biophysics Department then ceased to exist after only 31 years. BK continued to work at the laboratory, with the support of S. Zeki (FRS 1990). Zeki now works from the office that BK occupied during his tenure as head of department. When BK was in residence, the only luxuries were a leather chair and a small side room containing a film reader with a magnifier.

After his retirement, BK was more inclined to travel abroad, mostly accompanied by Rita, to give lectures to students, to meet old and new friends and to receive the many honours awarded to him. Despite his bad experiences as a youth in Leipzig, BK began to visit Germany in the postwar period on a regular basis. There he helped to inspire young German researchers. His first visit took place nearly 30 years after his emigration. W. Feldberg, a pharmacologist who worked with Sir Henry Dale, Brown and M. Vogt (FRS 1952) on the

ACh hypothesis after his emigration from Germany in 1933, set up a fund for reciprocal visits between English and German physiologists and pharmacologists to promote scientific exchange after the war. During his first visit BK was hosted by R. Stämpfli in Homburg/Saar and by H. Schaefer in Heidelberg. BK was also a regular guest at the Lindau meetings of Nobel laureates and students. He was always a star on these occasions—many students embarked on careers in neuroscience after hearing him speak. In 1982 BK became a member of the 'Orden pour le Mérite', an order that was founded in 1842 by a Prussian king and re-established in 1952. At present this order comprises 30 German and 30 non-German scientists and artists. The laudation for BK was given by W. Reichardt, the then director of the Max Planck Institute for Biological Cybernetics in Tübingen, where BK was a member of the scientific advisory board. BK responded with a polite address of acceptance, thanking the Orden for the honour of being elected and referring to himself as a 'hybrid' of a German and a non-German member.

About 100 friends and pupils 'who had learnt so much from him at the Department of Biophysics at UCL during the years 1952–1984' gathered in London to honour BK. He was delighted, as can be seen in photographs taken by J. Heuser. These show BK—with his characteristic shy smile—in the company of Fatt, del Castillo, Miledi and Eccles.

BK also accepted an honorary MD from the medical faculty of the University of Leipzig; this was awarded in 1990. In his memory a stone with a bronze plate was later placed in the garden of the University Hospital by the 'Bund der Albertiner', a union of former pupils of BK's secondary school, and a street in Leipzig was named after him.

In 1993, BK gave the Fenn Lecture (41) at the World Congress of International Union of Physiological Sciences (IUPS) in Glasgow, Scotland that was devoted to 'integrative physiology'. He reviewed the evidence for quantal secretion and exocytosis and, typically, noted that 'if cell physiologists didn't elucidate basic mechanisms in detail, then integrative physiology would have nothing to integrate'.

His last travel abroad with Rita was for a holiday in the Black Forest in 1994, after which Rita became too frail to travel. From 1997, Rita was cared for in a nursing home in Stanmore. BK took the bus to visit her every day, often reading excerpts from the novels of Jane Austen to her. He was still attending his UCL office daily, reading and critiquing preprints sent to him by his colleagues at UC or by former students, sometimes acerbically.

After Rita's death on 7 January 1999, BK's interest in most pursuits waned. However, he still visited his office several times each week, and was supported and helped by members of the Physiology Department, notably S. Page and S. Zeki, who also arranged his 90th birthday celebration at the Athenaeum. He lived alone in his house in Kenton Gardens, where he was cared for by a local lady, by his friend Oskar Hill, by J. Nicholls, who visited him regularly when he came from Trieste, and by his sons David and Jonathan. In January 2003, BK was hospitalized at St Vincent's in London. He died peacefully in April of that year. An era of biophysics has ended, and a teacher and a mentor to several generations of physiologists is now with us only in our hearts and memories.

BK's lasting impact on physiologists and on science

BK left plenty of questions for subsequent generations: the molecular details of release and the postsynaptic action of transmitters, the identity of the molecules that facilitate the influx

of calcium ions, how calcium and its sensors promote the coalescence of vesicles with the axon membrane, and how binding of a ligand opens an ion channel. Those who work on these questions admire BK's achievements and may see him as Horace saw himself:

> No, I shall not die completely: beyond the grave, the noblest part of me shall live, and for the world my name will grow… .

HONOURS AND AWARDS

Prizes and medals

1934	Siegfried Garten Prize, University of Leipzig
1965	Feldberg Foundation Award
1967	Copley Medal of the Royal Society
	Baly Medal Royal College of Physicians
1969	Knighthood
1970	Nobel Prize in Physiology or Medicine (jointly)
1989	Cothenius Medal Academia Leopoldina Halle
1990	Ralph W. Gerard Prize, Society for Neuroscience

Membership of academies and learned societies

1952	Fellow of the Royal Society
1961	Fellow of University College London
1968	Fellow, Royal Society of Physicians
	Foreign Member, Royal Danish Academy of Science and Letters
	Member, Accademia Nazionale Lincei
1969	Foreign Associate, American Academy of Arts and Sciences
1976	Foreign member, National Academy of Sciences, USA
1977	Hon. Member, Japanese Pharmacological Society
1978	Associate Member, EMBO
1982	Foreign Member, Orden Pour le mérite für Wissenschaften und Künste
1985	American Physiological Society

Honorary doctoral degrees

1971	Hon. DSc University of Southampton
	Hon. DSc University of Melbourne
1980	Hon. DSc Cambridge University
1979	Hon. PhD Weizmann Institute
1990	Hon. MD University of Leipzig

Named lectures

1958	Herter Lectures, Johns Hopkins University
1961	Dunham Lectures, Harvard University
1962	Croonian Lecture, Royal Society
1967	Sherrington Lecture, University of Liverpool
1985	Bayliss and Starling Memorial Lecture
1993	Fenn Lecture, IUPS, Glasgow

Other appointments

1938	Beit Memorial research fellowship
1939	Carnegie Research Fellow
1942/43	Pilot Officer Bernard Katz, Royal Australian Air Force
1946	Assistant Director of Research, Henry Head Research Fellow of the RS at UCL
1950	Appointed Reader in Physiology
1952	Professor of Biophysics at University College
1957–63	Editor, *Journal of Physiology*; Chairman 1961–63
1967–77	Member of Agricultural Research Council
1968–76	Biological Secretary of Royal Society and Vice President

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