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Obituary: Sir Bernard Katz (1911–2003)

Bernard Katz died peacefully on April 20th, aged 92. His genius was in the design and execution of experiments which provided remarkable insights into the mechanisms of synaptic function.

Early life

BK, as he was known by his scientific colleagues, was born in Leipzig in 1911 of stateless Russian Jewish parents. His father was born in Mogliev on Dnjepr in what is now the Ukraine, and left there for Germany following unrest at the time of the Russo-Japanese War in 1904. His family ancestors were Jews of Eastern European Ashkenazi origin. The surname Katz is an abbreviated form of the Hebrew words *Cohen Tsedek*, indicating a group of priests that claimed to be descended from Moses's brother, Aaron.

Katz was a brilliant student at school, winning prizes and skipping years to higher grades, although surprisingly he did not do science subjects. This inclined him towards a career in philosophy. Language masters had a considerable impact on his intellectual development, and made him 'treat words and phrases with respect and to use the language as a precision tool', a gift that was later to become one of the hallmarks of his scientific papers. At this time in his secondary education he became addicted to chess, which remained an obsession until it was replaced by neurophysiology. Katz attended some public lectures at the University of Leipzig towards the end of his secondary education, and these, together with the poor prospects of his father at that time, determined that he should to medicine.

The introduction of Katz to the natural sciences on entering the medical school in April 1929 had a tremendous effect, leading him to reject a career in philosophy. He comments that 'I felt that it was fascinating that one could make accurate and repeatable measurements of

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electrical excitability on living tissues and express the results by a simple mathematical equation', so that by 1930 he was already attracted to neurophysiology. This was such that following the preclinical exams in 1933 he began experimental research on muscle in the Physiological Institute, later published in *Pflugers Archiv*. In this work he found that stretching frog muscle leads to a change in the electric impedance of the muscle.

By 1932 it was obvious to Katz that Germany was not a place for a Jew so he began plans to emigrate following completion of the MD. It was fortunate that his grandfather, David Katz, had grandchildren who had already emigrated to Milan, New York and London. These were able to provide Katz with some support. He determined, on reading the papers and correspondence of A. V. Hill in Nature, that he would go to London and see if Hill would accept him for a research degree at University College. He arrived in London in February, 1935, following the award of an M.D. in November, 1934, with four pounds in his pocket. Hill accepted Katz, as an 'experiment', and although still stateless, the period in that laboratory between 1935 and 1939 were, Katz remarked 'the most inspiring period of my life'. This time marks the beginning of his research career.

Research highlights

1935–1936: INVESTIGATIONS ON NERVE EXCITABILITY

John Langley and Keith Lucas initiated the studies of A.V. Hill on the biophysics of nerve and muscle at Cambridge in 1909, and Hill in turn supervised the first research of Bernard Katz on electrical excitation and conduction of the nerve impulse at University College London in 1935. During this period Katz joined Hill and Donald Solandt, from Toronto to work on electrical excitation and conduction of the nerve impulse. Hill had developed, in 1933, a theory of electrical excitation and accommodation which assimilated a large number of observations. The first paper by Katz was in collaboration with Hill and Solandt on the extent of nerve excitation by alternating currents, published in the Proceedings of the Royal Society (1936). His next paper, in the same year, presented experimental work of his own in which he used for the first time a nerve—muscle preparation, namely the frog isolated gastrocnemius—sciatic nerve. With this preparation he investigated Hill's theory of excitation in order to provide an index for the duration of maintained nerve excitation.

1936–1939: MAGNESIUM IONS BLOCK NEUROMUSCULAR TRANSMISSION

The Cambridge zoologist Carl Pantin, who had acted as a guide to A.L. Hodgkin's studies, was responsible for the first research by Katz on neuromuscular transmission. This work showed, in 1936, that magnesium ions could block neuromuscular transmission in crabs. By 1939 Katz was using the frog isolated sartorius-nerve preparation after curarization to confirm the work of Gopfert and Schaefer (1938). This indicated the existence of a small non-conducted potential change at the myoneural region, which reached a maximum 4 msec after arrival of the nerve impulse, and then fell at a slow rate, similar to the electrotonic potential. Katz comments that at this time (in 1938):

> *The events that influenced my own experimental plans came from several directions: the single axon approach which I learned from Alan Hodgkin, the rather advanced electronic and oscillograph techniques introduced into our laboratory by Otto Schmitt, and the discovery by Dale and his colleagues of chemical transmission at the neuromuscular junction. Certainly, the work I was doing in 1938 to 1939, before I joined Eccles in Australia, was in line with these tendencies and had little connection with Hill's personal research.*

1940–1941: THE TIME COURSE OF TRANSMITTER ACTION IS VERY BRIEF COMPARED WITH THAT OF THE ENDPLATE POTENTIAL

During these two years Katz worked full time at the Kanematsu Institute in Sydney Hospital, affiliated with the University of Sydney. It was natural that he should have wanted to continue research on the 'small non-conducted potential change' or endplate potential, using the frog sartorius-nerve preparation, when he joined Jack Eccles and Stephen Kuffler at the Institute in 1939. Rather than continuing work on the innervation zones of the cat's soleus muscle with Eccles, Katz did the following:

> *I ganged up with Stephen Kuffler, and I was very pleased when we succeeded in getting hold of some nice Australian tree fogs whose sartorius muscles proved to be very suitable for the experiments we wanted to do, and this kept me busy and moderately happy for two years* (Katz, 1986).

The work of Katz, Kuffler, and Eccles in Sydney in 1940 and 1941 marks the beginning of a new era in synaptic physiology after the one begun 50 years earlier by Langley and Sherrington. It is characterised by the use of progressively more sophisticated electrical techniques to probe the mechanism of synaptic transmission. The first experiments of Eccles, Katz, and Kuffler did not involve test-conditioning volleys to estimate the time course of the excitatory state as Eccles had learnt from Sherrington some 15 years earlier, but rather concentrated on the properties of the extracellular signs of the endplate potential made subthreshold by a suitable dose of curare. They showed, using an analysis provided by A.V. Hill, that the time course of the underlying transmitter action lasted for only a few msec. As Eccles, Katz, and Kuffler stated:

Thus it seems that most of the declining phase of the e.p.p. is a passive decay of a negative membrane charge after the depolarizing agent has ceased to act. The earlier suggestion, therefore, that the decline of the endplate potential follows the time course of a passively decaying electrotonic potential is confirmed.

They stated further:

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

In 1941 Katz and Kuffler showed that the amplitude of the endplate potential recorded with an extracellular electrode from the frog neuromuscular junction is affected by calcium ions. However they were not to know that in 1940 Feng, working in China during the Sino-Japanese war, had already suggested that calcium may exert its effect on transmission by altering the amount of transmitter released.

At this time (1941) Katz became an Australian citizen and a British subject, so that he was no longer stateless at the age of 30! He then left the Kanematsu and enlisted in the Royal Australian Air Force in which he served until the end of the war as a radar officer, often in rather dangerous circumstances in places like New Guinea. The last year of the war was spent in more congenial surroundings in the Radiophysics Laboratory at the University of Sydney, with colleagues who were laying the foundations of radio astronomy and so placing the University at the forefront of this new discipline.

1946–1949: THE ACTION POTENTIAL IS DUE TO AN INWARD FLUX OF SODIUM IONS

Katz returned to Hill's laboratory in 1946 and spent a year working on the electrical properties of muscle fibres, discovering the phenomenon of anomalous or inward rectification. Then in the summer of 1947 he began a very fruitful collaboration with Alan Hodgkin on the squid axon, which resulted in their classical paper showing that the overshoot of the action potential is due to an influx of sodiium ions. He also participated in the first voltage—clamp experiments on the squid axon which were destined to be published as the first paper in the classic series by Hodgkin and Huxley that gave a quantitative account of nerve excitability.

1950–1954: THE DISCOVERY OF MINIATURE ENDPLATE POTENTIALS AND THE QUANTAL HYPOTHESIS FOR TRANSMITTER RELEASE

The introduction of the microelectrode by Ling and Gerard in 1949 prompted Katz to return once more to his first love, the frog muscle- nerve preparation. His first research in 1950 was to confirm, with Paul Fatt, the earlier work carried out in Sydney with extracellular electrodes, that the endplate potential alone initiated the muscle action potential. They also showed that this endplate potential arose due to the action of transmitter opening pores in the membrane which allowed the influx of ions.

A discovery was also made in 1950 that was destined to revolutionize our understanding of neurotransmitter release and earn Katz the Nobel Prize in 1970. This was the existence of 'biological noise' when recording the endplate potential at the amphibian neuromuscular junction with an intracellular microelectrode. Although the endplate potential in response to a nerve impulse had been identified in 1938, it was not possible to observe spontaneous endplate potentials without intracellular microelectrodes. The amplitude-frequency distribution of the spontaneous potentials was approximately Gaussian, although Fatt and Katz did note in 1952 that:

. . . there is an indication of several discharges of about twice the mean amplitude, and of one isolated discharge of three or four times the mean size.

They attributed this to the coincidence of two (or three) unitary discharges that could not be resolved given that detection was only possible down to 5 msec; the calculated chances of units occurring at such small intervals apart supported their conclusion. This question concerning the composition of the unitary discharges is still a matter of great interest.

It was natural to consider if the endplate potential (epp) was composed of these spontaneous unitary discharges. In 1954 Del Castillo and Katz showed that this was likely to be the case. They determined that the amplitude-frequency distribution of the endplate potential under conditions of low transmitter release could be built of units whose mean size and amplitude distribution were identical to those of the spontaneous unitary discharges. They concluded that:

statistical analysis indicates that the end-plate potential is built up of small all-or-none quanta which are identical in size and shape with the spontaneous occurring miniature potentials.

This gave rise to the 'quantal hypothesis' namely that the spontaneous unitary discharges or miniature endplate potentials (mepps) represented a quantum of transmitter release and the epp multiples of this quantum. Furthermore, 'suppose....that there are within the terminal area some 100 discrete "patches' concerned with the release of ACh. If these terminal structures have a special tendency to spontaneous excitation, then our observations would be easily understood'. So the concept of discrete zones for the secretion of transmitter had a physiological basis.

The possibility that the probability of release of such mepps might be increased on arrival of the nerve impulse was next examined by del Castillo and Katz. Motor-nerve terminals were depolarised with an external electrode and a greatly accelerated discharge of miniature epps noted. Unlike the amplitude of the miniatures, their frequency of discharge during the depolarisation was dependent on the calcium and magnesium concentration, dropping to nearly zero when the magnesium was increased and being restored to normal when calcium was added. These observations gave rise to the concept that calcium controls the probability of discharge of miniature epps under a depolarisation as a consequence of its interacting with a carrier molecule in the terminal, leading to the production of a miniature epp. The scheme is spelt out and is described in the words of del Castillo and Katz as follows:

> *On this hypothesis, the common step in spontaneous as well as evoked activity is the release of an active 'carrier molecule' (X′) which transports, or allows the passage of, a large number of ACh ions and leads to the production of a miniature e.p.p. There are different ways in which X′ can be formed: (i) from a CaX compound which is specifically acted on by the nerve impulse and transformed to Ca + X′, (ii) from other inactive precursors (X) which may change to X′, spontaneously, due to thermal activity. Only the first of these resources is blocked by Mg, or by Ca deficiency.*

This paragraph gives the first statement concerning a calcium sensor in the nerve terminal for transmitter release. Several points might be noted. First, the formation of the active molecule X′ involves the prior formation of the complex CaX; second, that this complex is formed under a depolarisation; and third, that X′ may form spontaneously as a consequence of some thermal activity in the terminal. It is not made explicit where X′ is, on the outside or the inside of the terminal, or in the membrane acting as a carrier? The model has, however, proved to be one of the most prescient in the history of neuroscience.

1956–1959: THE VESICLE HYPOTHESIS IS PROPOSED

In 1956 del Castillo and Katz enunciated the vesicle hypothesis, attributing quantization of transmitter release to its association with synaptic vesicles. In their own words:

> *Recent electron microscope studies (Robertson, 1956) have shown that the motor nerve terminals contain a fairly dense population of microsomes, granules or vesicles, of less than 0.1 micron diameter, which may well be the intracellular corpuscles to which ACh is attached. It has been known since Loewi's investigations that most of the ACh which is present in 'homogenised' nerve tissue can only be extracted into an aqueous solution after chemical destruction of the cell protein. It appears then that the discharge of ACh from a nerve terminal requires the disruption of more than one diffusion barrier: first the release from its intracellular attachment, and secondly a passage through a nerve membrane. One might suppose that when a 'critical' collision occurs between an intracellular ACh-carrier and the membrane of the nerve terminal, the two barriers are opened simultaneously and the ACh-contents of the carrier particle are suddenly discharged. This picture, though purely speculative, is nevertheless in accord with recent experimental findings; it takes account of the evidence discussed below that the release of ACh from nerve terminals occurs in multi-molecular units or 'quanta' and of the evidence, already cited, for the bound state of intracellular ACh content.*

The mechanism by which the 'contents of the carrier particle are suddenly discharged' is perhaps the major focus of research on neurotransmitter release at the present time.

1960: THE IDEA OF 'DISCRETE ZONES' OF MEMBRANE SPECIALIZATION AT SYNAPSES FOR TRANSMITTER RELEASE

The idea of discrete zones for the secretion of transmitter at nerve terminals, arrived at on the basis of physiological experiments mentioned above, was given substantial impetus by Katz, together with Birks and Huxley, on the introduction of the electron microscope for the examination of end-plate structure in 1960. This revealed synaptic vesicles in the nerve terminal, supporting the 'vesicle hypothesis' that the miniature units or quanta are due to the prepackaging of transmitter in vesicles. Furthermore, the ultrastructural observations of these authors that 'one often sees the vesicles concentrated in certain well-defined areas focussed on a dense zone of the axon membrane directly opposite a postsynaptic fold', which they referred to as 'special zones of the axon membrane', introduced the idea that such zones were the 'discrete zones' from which quanta are released. This gave rise to the 'active zone hypothesis', namely that these zones constitute the sites of quantal transmitter release.

1966–1970: CALCIUM MUST ENTER THE NERVE TERMINAL TO TRIGGER TRANSMITTER RELEASE

del Castillo and Katz, in their 1954 model of the calcium-sensor for transmitter release, had not made explicit where the calcium-sensor was to be found. Indeed their specification of the molecule, X′, that was ultimately responsible for secretion as a carrier molecule, implied that it carried the unit of transmitter across the nerve terminal membrane in the release process. The fact that X′ was formed from CaX did not make clear where the CaX itself was formed. Experiments performed in the mid-1960s by Katz and Miledi showed that either Ca^{2+} or

$Ca^{2+}X$ had to pass into the nerve terminal from the outside. With the discovery of tetrodotoxin it was now possible to depolarise nerve terminals with a focal electrode placed at a site on the nerve terminal without generating action potentials, and record the consequent evoked release of units of transmitter with an intracellular electrode. When this was done at the frog motor endplate it was noted that the minimal latency at which a transmitter unit was released increased with the duration of the depolarizing pulse. This was regarded by Katz and Miledi as:

> *. . . evidence suggesting that entry into the axon membrane of a positively charged substance (external Ca^{2+} ions or a calcium compound CaR^{+}) is the first step leading to the release of acetylcholine packets from the terminal.*

More direct evidence for this idea was obtained by direct depolarisation of the presynaptic membrane with an intracellular electrode in the presynaptic axon of the stellate ganglion of the squid and another intracellular electrode in th postsynaptic axon. Katz and Miledi showed in 1967 that in the presence of tetrodotoxin, short depolarizing pulses of the presynaptic membrane lead to a postsynaptic response that did not occur until the depolarisation was ended in the case of strong depolarizing pulses. This was the result to be expected if the strong presynaptic depolarisation prevented the influx of the positively charged Ca^{2+} or the CaR^{+}. The experiment did not distinguish however between the proposition that it was the entry of Ca^{2+} that was necessary for transmitter release or the calcium compound.

This led Katz and Miledi to comment that:

> *It may be that the opening of the external membrane 'gates' to Ca^{2+} or CaR^{+} is a transient event much briefer than the subsequent rise and fall of the probability of release. Or some calcium 'carrier' or 'receptor' only appears for a brief initial interval of time on the external surface of the axon membrane.*

Again it is clear that there is no way of distinguishing at this time between the two hypotheses of calcium movement through the nerve terminal or that of the preformed CaX into the terminal for transmitter release. The issue was crystallised by Katz and Miledi in 1970 in terms of testing what came to be known as the 'calcium hypothesis', namely that

> *. . . transmitter release is brought about by influx of external calcium ions through special membrane channels which are 'opened' by the depolarizing pulse.*

Thus at the end of the 1960s it was still not clear if the calcium hypothesis was correct or whether CaR^{+} had to enter the terminal.

An important experiment that showed at least that calcium did enter the terminal was then performed by Katz and Miledi. They showed that a regenerative response could be obtained from the presynaptic nerve terminal in the squid stellate ganglion in the presence of tetrodotoxin to block sodium influx and tetraethylammonium to block potassium efflux. Under these circumstances there was a long-lasting depolarisation with a duration of several hundred milliseconds after the depolarising stimulus. This response depended on the presence of calcium ions in the medium and was counteracted by magnesium and especially by manganese. It would seem in hindsight that the evidence was clear at this time that the regenerative response observed was due to calcium entering the nerve terminal. However, there was a complicating factor, namely that on removal of sodium ions the response eventually disappeared, leaving open the possibility that these ions contributed to the response.

In a further investigation of the role of calcium in transmitter release at the giant synapse in the stellate ganglion in 1970, Katz and Miledi measured the size of the postsynaptic response for a given presynaptic depolarisation, all in the presence of tetrodotoxin to block the generation of impulses. From this work they determined that at constant external calcium concentration and varying voltage a 'permeability coefficient' for calcium movement across the terminal might be derived, that changes with voltage. However this work presupposed the calcium hypothesis without proving it.

1970–1971: THE OPENING OF SINGLE ACETYLCHOLINE RECEPTOR CHANNELS

The study of the physiological action of single acetylcholine receptors began in 1970 with the discovery by Katz and Miledi of membrane noise at the endplate in response to the steady action of acetylcholine from a micropipette. They hypothesised that during such a steady application:

> *the statistical effects of molecular bombardment might be discernible as an increase in membrane noise, superimposed on the maintained average depolarisation.*

This is what in fact they observed with an intracellular electrode. A simple relationship was then used that connects the size of the elementary voltage due to the opening of a single channel as a consequence of acetylcholine binding to a receptor to the average depolarisation and the root mean square value of its fluctuation. This gave a value for the elementary event of 0.29 μV. In 1971 the same authors determined the approximate time course of the elementary conductance change underlying the elementary event by recording the extracellular voltage fluctuations due to the bombardment of receptors with acetylcholine. This gave an average time constant of the elementary event of about 1 ms and a net charge transfer across the open channel of about 5×10^4 univalent ions, with a channel conductance of

about 10^{-10} Siemens. Such determinations were later to be confirmed with the introduction of the patch-clamp technique for observing the action of single channels directly.

Epilogue

When I finished writing my book *History of the Synapse*, from which much of the above is derived, I was left with the conviction that young researchers on the synapse should read all of Katz's papers. Bert Sakmann and David Colquhoun have also expressed the same sentiment. I hope that the above brief summary gives some indication of the unique capacity which Katz possessed for teasing out the details of synaptic function. It is, however, only by reading the papers that his genius for research shines through.

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