Eric A Barnard FRS (1927 -2018)

J. Oliver Dolly
International Centre for Neurotherapeutics, Dublin City University

Jeremy M Henley
University of Bristol

F. Anne Stephenson
University College London School of Pharmacy

Eric Albert Barnard died on 23 May 2018 at the age of 90. In the 1980's, Eric was one of the first researchers to apply molecular biological techniques to the study of proteins of the nervous system and was a pioneer and founding father of the discipline of Molecular Neurobiology.

Eric or "Prof." as those of us who worked with him referred to him, was born on July 2 1927. He had a non-standard entry into science after a very tough childhood (The Times, 2018). He was brought up in an orphanage in South London before being evacuated during the war to Cambridge. He left school at 16 and worked as a messenger boy for a firm of stockbrokers before National Service with the RAF. He studied for his A levels at night and won a scholarship to Kings College London. There he joined the laboratory of Professor James Danielli, famed for the Davson-Danielli model of cell membranes, to undertake his PhD studies. His doctoral work resulted in the publication of his first paper in Nature, "A cytochemical reaction for nucleoprotein" (Barnard and Danielli, 1956). It was at Kings that he met his future wife, Penny Hennessy.

After gaining his PhD from Kings in 1956, Eric moved with Penny to the United States. He held a Rockefeller Foundation Award to go to UCLA Berkeley, California for a year then moving to the State University of New York in Buffalo, becoming Professor in 1968 and Chairman of the Department of Biochemistry. At that time he was primarily a protein chemist and enzymologist. He became head of the Molecular Enzymology Unit at Buffalo and eventually, the Head of the Biochemistry Department. During this time, Eric published seminal works on the enzymes, hexokinase and ribonuclease and began his early forays into Neuroscience. He used electron microscopy to locate and quantify the protein receptor that responds to acetylcholine as well as the enzyme that degrades acetylcholine to switch off transmission of the signal (Barnard *et al*., 1971). He was elected a Fellow of the Royal Society in 1981. The citation for his election reads:-

"Distinguished internationally for his contributions to protein Chemistry and Neurochemistry. He pioneered affinity labelling by identifying His-119 in the active centre of ribonuclease-A and his sequence studies of this enzyme in vertebrates have illuminated protein evolution. He first purified native yeast hexokinase and has contributed much to our present knowledge of its structure and isoenzymes. He developed the use of labelled inhibitors to locate and quantify acetylcholinesterase and acetylcholine receptors in the ultrastructure of the nerve-muscle synapse. He was the first to purify the mammalian cholinergic receptor and is using it

to construct model synaptic membranes. These studies are leading him to an effective chemotherapy for muscular dystrophy".

Eric's focus moved away from enzymology and he became interested in the mammalian neuromuscular junction where he began to characterize in depth and quantify the components of nicotinic cholinergic neurotransmission, nicotinic acetylcholine receptors and acetylcholinesterase. With his colleague, Oliver Dolly, he applied methods and tools such as α-bungarotoxin that had been used to purify the nicotinic acetylcholine neurotransmitter receptor from electric organs of eel and ray fish, to isolate, the mammalian muscle nicotinic acetylcholine receptor. These studies complemented collaborative work with his wife on animal models of muscular dystrophy.

In 1975, Eric returned to the UK to the Department of Biochemistry at Imperial College, London to become the Joseph Rank Professor of Physiological Biochemistry. It was at Imperial that he applied molecular biological methods to study proteins of the nervous system. He recognized that because neurotransmitter receptors are often only a very small proportion of the total protein in brain, it would be extremely challenging to understand their molecular mechanisms of action since it was very difficult to isolate them in sufficient quantity and purity for detailed investigation of their structure and function. To circumvent this problem, in collaboration with Brian Richards who was then at Searle Research and Development, he exploited the Xenopus oocyte translation system pioneered by John Gurdon to demonstrate that oocytes injected with mRNA from Torpedo could synthesize nicotinic acetylcholine receptors that bound the snake α neurotoxins (Sumikawa *et al*., 1981). In collaboration with Ricardo Miledi, then at University College, London, he demonstrated that not only were these receptors targeted to the oocyte membrane and surface expressed but that they formed functional, acetylcholine-gated ion channels (Barnard *et al*., 1982). This was a landmark paper. In subsequent collaborations with David Brown, Trevor Smart and Andy Constanti, Eric went on to show that oocytes injected with total brain mRNA synthesized, amongst others, functional inhibitory $GABA_A$ and glycine receptors and excitatory glutamate receptors (Houamed *et al*., 1984). This methodology changed the way in which both ligand-gated and voltage-gated ion channels were studied. Almost all electrophysiology laboratories exploited this method until further advances led to the direct expression of receptors in mammalian cell lines. A direct consequence of these pioneering oocyte expression studies was the cloning by other groups, without protein sequence information, of the cDNAs encoding AMPA and NMDA receptors amongst others ((Hollmann *et al*., 1989; Moriyoshi *et al*., 1991). Eric published the cDNA sequence encoding the Torpedo nicotinic acetylcholine receptor α subunit (Sumikawa *et a*l., 1982). At the time this was an intensely active and competitive field of study and several groups (John Pierre Changeux, Shosaku Numa and Stephen Heinemann) published sequence data almost simultaneously. These molecular neuroscience contributions transformed the study of the neurotransmitter receptors and ion channels.

In 1985, Eric was appointed the Director of an MRC unit in Cambridge taking over from Lesley Iversen who led the MRC Neurochemical Pharmacology Unit. Under Eric this became the MRC Molecular Neurobiology Unit where he continued to develop and apply innovative molecular methods to the study of neurotransmitter receptors and other key proteins of the nervous system. Perhaps the biggest success of the unit was the cloning of the $GABA_A$ receptor subunit cDNAs and the discovery of the ligand-gated ion channel superfamily. The

inhibitory $GABA_A$ receptor had been purified to homogeneity at Imperial College in 1982 in Eric's lab. with the aim of using protein sequence data to clone the receptor cDNAs. Unbelievable as it sounds today, the major stumbling block was obtaining partial amino acid sequence so that oligonucleotide probes could be designed to screen cDNA libraries to identify receptor cDNAs. Eventually, after several failed approaches, collaboration was established with Peter Seeburg and J. Ramachandran both then at Genentech in California. The collaboration was successful. In 1987, a major article that became a citation classic was published in Nature in which the amino acid sequences of the $GABA_A$ receptor α and β subunits were described (Schofield *et al*., 1987). The article was published concurrently with one from the group of Heinrich Betz describing the amino acid sequence of the glycine receptor (Grenningloh *et al*., 1987). Importantly, both groups uncovered a predicted amino acid homology between $GABA_A$ and glycine receptor subunits, predicted because both are ligand-gated anion channels. Unexpectedly, however, homology was also found with the nicotinic acetylcholine receptor, a ligand-gated cation channel. Thus the ligand-gated ion channel super-family was uncovered. A follow up paper described the existence of $GABA_A$ receptor subtypes (Levitan *et al*., 1988). Building on this success, Peter Seeburg went on to find the $GABA_A$ receptor γ2 which endowed the recombinant receptors with benzodiazepine sensitivity. These findings paved the way for the search for new anti-anxiety drugs that lacked the addictive properties of the benzodiazepines.

Eric retired from the MRC in 1992 but continued his research. Firstly, he became the Director of the Neurobiology Unit at the Royal Free Hospital and University College, London; latterly he was in the Department of Pharmacology, University of Cambridge where he enjoyed a successful collaboration with David Brown on the properties of purinergic P2X and P2Y receptors. He finally "hung up his lab. coat" at the age of 85.

Prof. was the recipient of many awards and prizes. Prominent of these were his election as a Fellow of the Royal Society and the Thudicum Medal of the Biochemical Society which is awarded to honour eminent scientists who have made outstanding contributions to Neurochemistry.

As described in his obituary in the Times (1), Eric was indeed the archetypal absent-minded Professor. To those of us who worked with him, he was always full of energy and totally focused on his science. So much so that famously, he once left his lab. to go to the airport only to realise that he had forgotten to collect something important, Penny, his wife! In pre-Powerpoint days, he was notorious for preparing his slides at the very last minute often making final changes which then necessitated obscuring parts of the slides with tape. Not optimal with respect to presentational skills!

Eric was an inspirational scientist who trained very many PhD students and post-docs. who went on to successful careers in their own right. He will be sadly missed.

Acknowledgments and conflict of interest disclosure

The authors have no conflict of interest to declare.

REFERENCES

Barnard, E.A. and Danielli, J.F. (1956) A cytochemical reaction for nucleoprotein. Nature 178, 1450-1453.

Barnard, E.A., Wieckowski, J and Chiu, J.H. (1971) Cholinergic receptor molecules and cholinesterase molecules at mouse skeletal muscle junctions. Nature 234, 207–209.

Barnard, E.A., Miledi, R. and Sumikawa, K. (1982) Translation of exogenous messenger RNA coding for nicotinic acetylcholine receptors produces functional receptors in Xenopus oocytes. Proc. Royal Soc. Lond B Biol. Sci. 215, 241-246.

Grenningloh, G., Rienitz, A., Schmitt, B., Methfessel, C., Zensen, M., Beyreuther, K., Gundelfinger, E.D. and Betz, H (1987) The strychnine-binding subunit of the glycine receptor shows homology with nicotinic acetylcholine receptors. Nature. 328, 215-220.

Hollmann, M., O'Shea-Greenfield, A., Rogers, S.W. and Heinemann S. (1989) Cloning by functional expression of a member of the glutamate receptor family. Nature 342, 643-648.

Houamed, K., Bilbe, G., Smart, T.G., Constanti, A., Brown, D.A., Barnard, E.A. and Richards, B. M. (1984) Expression of functional GABA, glycine and glutamate receptors in Xenopus oocytes injected with rat brain mRNA. Nature 310, 318–321. .

Levitan, E.S., Schofield, P.R., Burt, D.R., Rhee, L.M., Wisden, W., Kohler, M., Rodriguez, H., Stephenson, F.A., Darlison, M.G., Barnard, E.A. and Seeburg, P.H. (1988) Structural and functional basis for $GABA_A$ receptor heterogeneity. Nature 335, 76-78.

Moriyoshi, K., Masu, M., Ishii, T., Shigemoto, R., Mizuno, N. and Nakanishi S. (1991) Molecular cloning and characterization of the rat NMDA receptor. Nature 354, 31-37.

Schofield, P.R., Darlison, M.G., Fujita, N., Burt, D.R., Stephenson, F.A., Rodriguez, H., Rhee, L.M., Ramachandran, J., Reale, V., Glencorse, T.A., Seeburg, P.H. and Barnard, E.A. (1978) Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor super-family. Nature 328, 221-227.

Sumikawa, K., Houghton, M., Emtage, J.S., Richards, B.M. and Barnard, E.A. (1981) Active multi-subunit ACh receptor assembled by translation of heterologous mRNA in Xenopus oocytes. Nature 292, 862–864.

Sumikawa,K., Houghton, M., Smith, J.C., Bell,L., Richards, B.M and Barnard, E.A. (1982) The molecular cloning and characterisation of cDNA coding for the alpha subunit of the acetylcholine receptor. Nucleic Acids Res. 10, 5809–5822.

The Times (2018) Professor Eric Barnard Obituary, July 7.

Eric receiving the Thudicum Medal of the Biochemical Society at the University of Durham in 2008. Published with permission from Paul Sydney, Durham University.