ERIC A. BARNARD
2 July 1927 — 23 May 2018
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Elected FRS 1981

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Eric Barnard was a protein biochemist who played a leading role in the delineation of the molecular components of neuromuscular transmission and the emergence of molecular neuroscience as a scientific discipline. He began his career at King’s College London, moving to the State University of Buffalo, New York, in 1965 before returning to Imperial College, London, in 1975. In 1985 he became the Director of the Medical Research Council (MRC) Molecular Neurobiology Unit in Cambridge. Upon retirement from the MRC, he moved to the Royal Free Hospital in London where he continued as Director of Molecular Neurobiology, but in 1998 returned to the University of Cambridge (Department of Pharmacology) as Emeritus Professor. In 2014, at the age of 86, he finally retired from active research. Although Eric was elected FRS for his early pioneering work on the protein chemistry of enzymes and the nicotinic acetylcholine receptor, his seminal contribution, initiated during his time at Imperial, was the application of molecular biological methods to the study of many neurotransmitter receptors. With Ricardo Miledi FRS (and later David Brown FRS and colleagues), he developed the *Xenopus* oocyte system for the expression of receptors from total tissue mRNA. His was the first group to clone a neurotransmitter receptor subunit cDNA, the nicotinic acetylcholine receptor α subunit of *Torpedo marmorata*. This was followed by purification and subsequent cloning of inhibitory γ-aminobutyric acid (GABA)A receptor subunit cDNAs. This achievement, driven by Eric and aided by his collaborator Peter Seeburg, led to the discovery of the ligand-gated ion channel superfamily, the discovery of neurotransmitter receptor heterogeneity, and the development of concepts of receptor families and superfamilies. His pioneering work was pivotal for the foundation of modern central nervous system drug discovery.

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Early life

Eric Albert Barnard was born on 2 July 1927 in Greenwich, South East London, to Amelia and Albert Barnard, a builder. He had a younger sister, Hilda. Early life for the Barnard family and Eric in particular was hard. His father left home when Eric and Hilda were young, leaving his mother destitute, and left on her own, she found it hard to cope with two young children. As a result, Eric was placed in a Jewish children’s home in Norwood in South East London. His mother, Amelia, wanted to put Hilda into the same children’s home but instead she was mostly cared for by her uncle and aunt, Alfred and Alice. They wanted to adopt both Eric and Hilda but their mother blocked Eric’s adoption and eventually there was a court custody battle for both children. Hilda was duly adopted by her aunt and uncle while the court ruled that Eric had had enough upset in his life and that, aged 8, he should stay in the orphanage in Norwood where he was established. Despite living apart, Eric was able to maintain regular contact with his sister, at least in the short term, when he visited his aunt and Hilda. Although missing out on family life, academically he did well. He was strongly self-motivated and the only boy in the orphanage to pass the 11-plus examination, being awarded a scholarship for the Davenant Foundation Grammar School, which was then in Whitechapel, East London. The school had been founded in the seventeenth century by the philanthropist, Reverend Ralph Davenant, to educate the poor boys of Whitechapel. Eric’s scholarship resulted in the entire orphanage being given the day off to celebrate his success. When he was 12, the school was evacuated to Cambridgeshire, and Eric was placed in the home of a spinster who was not fond of children, so once again family life eluded him. Contact with his aunt and sister was lost around this time, but after the war, his aunt enlisted the help of the Salvation Army to find him, and eventually after two years the family were reunited.

Eric left school at 16 and went to work as an outdoor clerk running messages at a firm of stockbrokers in the City of London. He did National Service with the RAF, serving in Singapore and Malaysia, and afterwards, whilst working full time, he attended night school to study for his A-levels. He gained a place at King’s College London (KCL), where he undertook both his undergraduate and postgraduate studies (figure 1). His PhD in Biochemistry and Cell Biology, entitled ‘Studies on the chemical basis of the blocked diazonium reaction in cytochemistry’, was awarded in 1957. It was funded by the British Empire Cancer Campaign and undertaken in the laboratory of Professor James Danielli (FRS 1957), famed for the Davson–Danielli model of cell membranes. The research yielded his first publication, a letter in Nature (1)*. Eric’s colleagues at KCL included Rosalind Franklin and Maurice Wilkins (FRS 1959), and in a subsequent paper, Eric thanked Wilkins and also John (later Sir John) Randall FRS for the use of instrumental facilities in the MRC Biophysics Research Unit at KCL (2). It was during his PhD studies that he met Penny Hennessy, who was working in the same laboratory. After only eight weeks of courtship, Eric and Penny were married. Permission for their marriage was sought from Danielli, who reportedly approved with the proviso that if Penny became pregnant, she would have to leave. In fact she did become pregnant with her first child but stayed in post, not informing Danielli until after she had completed her studies. Penny and Eric went on to have four children, two boys, Matthew and Guy and two girls, Lisa and Sara.

* Numbers in this form refer to the bibliography at the end of the text.
Figure 1. Eric in 1950, the time of his undergraduate and PhD studies at King’s College, London.

CAREER

On completion of his PhD, Eric was awarded a Nuffield Foundation Fellowship to continue his studies at KCL. In 1960, he was granted a Rockefeller Foundation Award and spent two years at the Biochemistry and Virus Laboratory, University of California, Berkeley, before returning to KCL as a lecturer. He returned to the USA to take up an associate professorship in Biochemical Pharmacology at the State University of New York in Buffalo, New York and there became professor in 1968 and, in 1969, Chairman of the Department of Biochemistry. Whilst at Buffalo, he enjoyed two six-month sabbatical leaves funded by a Guggenheim Fellowship; the first was spent at the MRC Molecular Biology Unit in Cambridge, UK, and the second, in 1973, in the laboratory of Jean-Pierre Changeux at the Institut Pasteur in Paris, France.
In 1975, at the invitation of Sir Brian (later Lord) Flowers FRS, the Rector of Imperial College London, Eric was tempted to return to the UK. This was despite one of his colleagues commenting ‘Rats leaving the sinking ship I understand, but rats returning to the ship I do not!’ At Imperial, Eric held the first J. Arthur Rank Professorship of Physiological Biochemistry in the Department of Biochemistry, which had been founded by Sir Ernst Chain FRS in 1973. When Eric joined in 1975, it was headed by Brian Hartley FRS, who had consolidated the work of Chain by establishing a modern biochemistry department which combined the maturing field of protein chemistry with powerful new methodologies for studying structures of proteins. These included a high-mass biological mass spectrometry facility, the first of its kind in the world, together with researchers employing the rapidly emerging discipline of molecular biology. Eric succeeded Hartley as the Head of Department in 1979. He was elected FRS in 1981. The citation for his election read:

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 was the Head of the Biochemistry Department at Imperial until 1985, when he became the Director of the new MRC Molecular Neurobiology Unit in Cambridge. The Unit occupied space vacated by the closure of the MRC Neurochemical Pharmacology following the move of its Director, Lesley Iversen FRS, to become the first Director of the Merck, Sharp & Dohme Neuroscience Research Centre in Hoddesdon, UK. The MRC Molecular Neurobiology Unit reflected the emergence of molecular neurobiology (later termed molecular neuroscience) as a scientific discipline, pioneered at an international level in the 1980s by Eric and his ground-breaking research at Imperial. His appointment in Cambridge created much media interest, making headline news on BBC2’s Newsnight programme, with Eric being interviewed in his laboratory on the fifth floor of the Biochemistry Department at Imperial by Jenni (later Dame Jenni) Murray.

In 1992, at the age of 65, Eric had to retire from his MRC Directorship. A Festschrift was held in his honour at Robinson College, Cambridge, in 1992 (Deuchars & Thomson 1993; Usherwood 1993). His research, however, continued. Prior to his retirement from the MRC, Eric had been a Visiting Professor at the Royal Free Hospital (now part of University College London), and he became Director of the Wellcome Trust Molecular Neurobiology Research Group at the Royal Free. Subsequently, in 1998, he returned to Cambridge as Emeritus Professor in the Department of Pharmacology. His laboratory remained active, enjoying a fruitful collaboration with David Brown FRS, funded primarily by the Wellcome Trust. He finally retired from active research in 2014 at the age of 86.

**Early research: Eric the enzymologist**

Eric’s PhD thesis, published in 1957, was entitled ‘Studies on the chemical basis of the blocked diazonium reaction in cytochemistry’. The aim was to develop methods to label proteins
irreversibly by covalent reactions. Specifically, this was via reaction of accessible amino acid side-chains of proteins with diazonium hydroxide followed by reaction with tetrazotized dianisidine so that the labelled proteins could be visualized under the light microscope. This was a major advance, as the localization of proteins inside the cell had been largely unknown. Today the study seems very non-specific since diazonium hydroxide will react with any available tyrosine, histidine, and maybe tryptophan amino acid side-chains, of any of the many proteins found inside cells. Nevertheless it provided a means to visualize ‘proteins’ and revealed the potential for studying their cellular and subcellular distributions, their identity and their interactions at the cellular level—all of which are important for understanding protein function and the working of cells. The research resulted in Eric’s first publication in *Nature* (1). Curiously, Eric’s PhD thesis was submitted by Albert Eric Barnard whereas his *Nature* paper and all subsequent publications were by E. A. Barnard.

The development of the cytochemical approach to the study of proteins was a forerunner to much of Eric’s ensuing research career. In the early days, whilst establishing his own independent laboratory at KCL, his focus was on enzymes and the development of chromogenic, covalent labels (2, 3). Subsequently, labelling with radioisotopes, specifically tritium-labelled acetic anhydride employed as an acylating reagent, permitted localization but, even more important, cytochemical quantification of proteins (5). Further refinement described the use of substrate analogues to label (6) and affinity label (8) amino acids at the active site of ribonuclease. The latter facilitated the crystallographic determination of the structure of bovine pancreatic ribonuclease (*Avey et al. 1967*). Additional advances from this period included the use of covalent affinity labelling with radioactive substrates to study and quantify the protease and esterase enzyme activities in mast cells at the light and, now, electron microscopic level, i.e. quantitative autoradiography (9, 10). Further aspects explored the role of sulfhydryl side-chains in the dissociation of the enzyme hexokinase (e.g. (11)), and insights into protein-folding mechanisms via the study of seven species of ribonuclease (15).

**THE NEUROMUSCULAR JUNCTION: ACETYLCHOLINESTERASE**

Information transfer between skeletal muscle and nerve, and indeed between neurons in the brain, is discontinuous since the majority of these excitable cells are not in direct contact. The neurohumoral transmission developed by Otto Loewi (ForMemRS 1954) and Sir Henry Dale (FRS 1914) in the early twentieth century explained how communication between adjacent excitable cells occurs. Thus the electrical signal from an activated neuron to the recipient cell is mediated via the release of chemical messengers that interact with the receiving cell at specialized areas which became known as the neuromuscular junction for nerve–muscle communication or synapses in the brain. In the 1960s, it was well established that the chemical messenger or ‘neurotransmitter’ at the neuromuscular junction was acetylcholine and that the enzyme, acetylcholinesterase, degraded acetylcholine. How acetylcholine worked at the molecular level was unknown.

Eric’s interest in molecular mechanisms of neuromuscular transmission began during his time at KCL, where he started to apply the knowledge he had acquired for the study of molecular forms and active sites of enzymes to the proteins involved in nerve–muscle communication. His earliest studies were aimed at applying his innovative cytochemical techniques to the radiolabelling of the enzyme acetylcholinesterase at the motor end plate
Figure 2. Eric and Penny by the side of the Mazurian lakes in Poland on one of their holidays with his friend and collaborator, Kazimier Ostrowski (photograph provided by Dariusz Gorecki).

Eric’s first paper in the field was co-authored with Kazimier Ostrowski from Poland as early as 1961 (4). Eric and Ostrowski became long-term colleagues and friends, and Ostrowski and other co-workers from Poland were frequent visitors to Eric’s laboratories both in the USA and later in the UK. Further, in a memoir written to honour Ostrowski, Eric credited him with converting Penny and himself from a mainly sedentary city life to the pursuit of the ‘outdoor life’ (figure 2).

How acetylcholine released by the nerve cell elicits a response in the receiving muscle cell was not known. The hypothesis was that there should be a ‘receptor’ for acetylcholine expressed on the surface of the receiving muscle cell at the neuromuscular junction. This receptor would, it was postulated, somehow convert the chemical messenger into an electrical signal that would propagate along the muscle fibre and lead to muscle contraction. The nature of this ‘receptor’ was much debated, with suggestions that it was a proteolipid or that the
protein acetylcholinesterase itself was the receptor. In the absence of labels with which to
determine the putative receptor, Eric focused on acetylcholinesterase. He extended his earlier
studies to devise a method to label covalently the active site of acetylcholinesterase with the
inhibitor, di-isopropylfluorophosphate (DFP). He, with Ostrowski and other co-workers, used
radioactive [\textsuperscript{32}P]DFP, rather than [\textsuperscript{3}H]DFP used in their initial study (4), to make the first
application of quantitative ligand-binding autoradiography at motor end plates (7, 12, 13). This
meant that he was able to measure the absolute numbers of acetylcholinesterase molecules and
show that there is a fixed number at the mature junction depending on the muscle and fibre
and fibre type (11). At higher resolution in the electron microscope, Eric’s studies revealed that synaptic
acetylcholinesterase molecules at the neuromuscular junction are located over the full extent
of the highly folded muscle membrane at a constant density of 3000 per µm\textsuperscript{2} (12).

Through the years, Eric expanded his study of acetylcholinesterase to include investigation
of its molecular forms, their regulation and physiological significance in chemical
transmission at normal and denervated neuromuscular junctions, and also in a chick animal
model of muscular dystrophy. Much of the acetylcholinesterase work was in collaboration
with Israel Silman (Weizmann Institute, Israel) (21) and later, with one of his former PhD
students at Imperial College, Karl Tsim (Hong Kong University of Science and Technology,
Hong Kong) (40). Eric’s muscular dystrophy studies encompassed exploring novel ways
to delay the time of onset of symptoms (18): the significance of expression profiles of
acetylcholinesterase and pseudocholinesterase (21) and the contribution of differential muscle
fibre types in the development of disease (27,32), all in the chick model of muscular dystrophy.
With his wife, Penny, in later work, they switched to studying the mdx mouse, an X-linked
myopathic mutant animal model of human Duchenne muscular dystrophy. They reported
that the mdx mutation is located within the mouse Duchenne muscular dystrophy gene. Further, they went on to show that a single base substitution within an exon caused premature
termination of the polypeptide chain, thus explaining the impairment of muscle contractions
in this animal model (41, 43).

**THE NEUROMUSCULAR JUNCTION: NICOTINIC ACETYLCHOLINE RECEPTORS**

Analogous to transmission at the mammalian neuromuscular junction and owing to the
work primarily of David Nachmansohn (1959), chemical transmission between the cells
of the electric organ of the electric eel, *Electrophorus electricus*, and of the electric ray
fishes, such as *Torpedo marmorata*, was known to be mediated by acetylcholine. In the late
1960s and early 1970s, evidence was accumulating towards the recognition that there were
nicotinic acetylcholine receptors in these electric organs, distinct from acetylcholinesterase,
and discrete, integral membrane proteins (Meunier et al. 1971; Miledi et al. 1971). Their
proteinaceous nature was aided by the discovery of the pharmacological effects of the snake
venom α-bungarotoxin, a pseudo-reversible, high-affinity nicotinic acetylcholine receptor
antagonist (Chang & Lee 1963). Eric exploited these properties by using [\textsuperscript{3}H]α-bungarotoxin
for autoradiographic localization of acetylcholine receptors at the neuromuscular junction (14,
16) (figure 3). His group, in collaboration with Edson X. Albuquerque, determined for the first
time the absolute numbers of nicotinic acetylcholine receptors at the neuromuscular junction
(10 000 per µm\textsuperscript{2} (17)). The receptors were localized at the crests of the postsynaptic folds of
each neuromuscular junction at a constant density and this was related to the motor end plate
Figure 3. Electron microscope autoradiograph of a section through a red fibre muscle end plate with $[^3]$H$\alpha$-bungarotoxin. Muscle end-plate-rich areas were dissected out, washed, fixed in OsO$_4$, dehydrated, and embedded in Epon–Araldite. Ultramicrotome sections were mounted on collodionized slides, stained with uranyl acetate, carbon-coated and overlaid with a monolayer of Ilford L4 emulsion before exposing to Kodak Microdol X film. The dark areas are the grains of $[^3]$H$\alpha$-bungarotoxin, which can be seen to label the folds at the neuromuscular junction. Outside this area, labelling is very low. Note the circular structures within the nerve axon, which are vesicles containing the neurotransmitter acetylcholine. ax, axon; m, muscle; pjf, post-junctional folds. Reproduced with permission from (16).

current evoked when acetylcholine was released. These findings were shown to hold equally in other muscles and species (17) and formed the foundation for subsequent modelling of neurotransmission at the neuromuscular junction.

Although $\alpha$-bungarotoxin was an invaluable tool for the localization and quantification of nicotinic acetylcholine receptors, it was not useful for protein purification because it was such a high-affinity irreversible ligand. The mode of action of a related $\alpha$-neurotoxin from the snake *Naja naja siamensis* was found to be similar to that of $\alpha$-bungarotoxin but, importantly, this $\alpha$-neurotoxin had a lower affinity for the receptor, which meant that it could be employed as an affinity ligand to isolate receptors using a technique known as affinity chromatography. First pioneered as a technique for isolating enzymes by Cuatrecasas *et al.* (1968), this involved allowing the molecule to be purified to bind to its ligand, which had first been attached to an insoluble polymer; after washing away all contaminants, the target molecule could be released by high concentrations of salt, change of pH or, optimally, high concentrations of a competing ligand. Before the advent of cDNA clones and *in vitro* synthesis of specific proteins, this was the only way to obtain relatively pure proteins. Jean-Pierre Changeux in France and Michael Raftery (FRS 1986) in the USA first used this to isolate nicotinic acetylcholine receptors from electric ray and electric eel fishes, respectively, both abundant sources of the receptor protein. Importantly, in addition, because the receptors were integral membrane proteins, they required detergents to extract them from the membrane bilayer; at that time, few integral membrane
proteins had been successfully detergent-solubilized with retained pharmacological activity. In collaboration with Oliver Dolly, Eric then used the same method to isolate for the first time nicotinic acetylcholine receptors from mammalian skeletal muscle (19, 20). That work had the added challenge that the acetylcholine receptors in muscle were only present at each tiny neuromuscular junction and therefore the concentration in muscle was extremely low compared with that found in fish electric organs. Nevertheless, purification was successful and the receptor was found to be similar to that of the electric organ in that it contained a major polypeptide chain of ca. 40 kDa, identified by affinity labelling as the acetylcholine binding subunit (19, 20). Importantly around this time, by reconstitution of the purified receptor protein into synthetic lipid bilayers, Changeux’s group amongst others established that nicotinic acetylcholine receptors contained an integral ion channel (reviewed in Changeux 2012). Thus within milliseconds of acetylcholine binding, the ion channel opens, sodium ions enter the muscle cell, the membrane depolarizes, and ultimately the muscle responds to the incoming signal from the nerve by contraction. It is now known that there are two types of muscle nicotinic receptors, embryonic and adult. Both are multi-subunit, acetylcholine-gated pentameric ion channels, comprising $\alpha_1\beta_1\gamma\delta$ and $\alpha_1\beta_\epsilon\delta$ subunits, respectively.

Eric extended the same affinity chromatography methods to isolate an $\alpha$-neurotoxin binding subunit from the chick optic lobe (24). This was controversial since at that stage there was no evidence for $\alpha$-neurotoxin antagonism of acetylcholine neurotransmission in the brain. However, in a collaboration with Susan Dunn and Michael Raftery at CalTech, USA, N-terminal micro amino acid sequencing revealed that the isolated brain receptor was different from, although homologous to, muscle nicotinic receptors (30). This receptor is now known as the $\alpha_7$ subunit, which forms the only functional, homomeric (five $\alpha_7$ subunits) brain acetylcholine receptor. See Lukas et al. (1999) for a full list of the nicotinic acetylcholine receptor gene family.

**Imperial College: the dawn of molecular neuroscience**

As described above, in 1975 Eric returned to the UK to become the Joseph Rank Professor of Physiological Biochemistry at Imperial College of Science and Technology in London. His research was focused into three main areas: the continued study of the structures and functions of the enzymes hexokinase and acetylcholinesterase; in collaboration with his wife, the molecular changes at the neuromuscular junction observed in the chick model of muscular dystrophy; and, with Oliver Dolly, who left Buffalo to join him as a lecturer at Imperial, he extended his work on the quantification and, eventually, the molecular properties of nicotinic acetylcholine receptors.

In parallel with these strands of research, Eric was one of the first to recognize that detailed structural studies could not be performed on native neurotransmitter receptors simply because of the small amounts of receptor protein that could be isolated, and because of the limitations of the biophysical techniques then available. Thus, along with pursuing a conventional biochemical approach to the study of receptor proteins, Eric took advantage of the ‘new technology’ of DNA cloning to circumvent the shortcomings of traditional protein chemistry methods, i.e. rather than isolating the receptor from the brain, the aim was to identify the complementary DNA (cDNA) or genes encoding the receptor; these could then be expressed in bacteria, yeast, insect or mammalian cells, yielding high concentrations enabling
the detailed study of their structures and their pharmacological and physiological properties. This saw the emergence of molecular neuroscience as a scientific discipline of which Eric was a leading international pioneer.

For anticipated future molecular studies it was necessary first to establish a method by which any potential cloned multi-subunit integral membrane proteins such as nicotinic acetylcholine receptors could be investigated. In collaboration with his friend and colleague from KCL days, Brian Richards, and Katumi Sumikawa, both at Searle Research and Development (Buckinghamshire, UK), he exploited the *Xenopus* oocyte translation system pioneered by John (later Sir John) Gurdon FRS to demonstrate that frog eggs (oocytes) injected with mRNA from the electric organ of the ray *T. marmorata* could synthesize nicotinic acetylcholine receptors that bound the snake α-neurotoxins (22). In partnership with the eminent electrophysiologist, Ricardo Miledi FRS, then at University College, London, in a landmark paper, he showed not only that these receptors were targeted to the oocyte membrane and expressed on its surface but that they could be studied electrically and be shown to be functional, acetylcholine-gated ion channels (figure 4) (23). The collaboration with Miledi floundered, but in a new collaboration with David Brown, Trevor Smart and Andy Constanti at the University of London School of Pharmacy, Eric went on to show that oocytes injected with total brain mRNA synthesized neurotransmitter receptors that are expressed in the brain, including functional, inhibitory GABA<sub>A</sub> and glycine receptors, as well as excitatory glutamate receptors (29). The oocyte translation methodology represented a seismic shift in the way in which both ligand-gated and voltage-gated ion channels were investigated. Very many electrophysiology laboratories subsequently exploited oocyte expression to study, in isolation, the pharmacological and biophysical properties of native, i.e. wild-type, receptors and ion channels and, once cDNA clones were identified, mutant receptors created by protein engineering methods. In addition, oocyte expression permitted cloning in the absence of protein amino acid sequence information. Two important examples are the cloning of cDNAs encoding α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) excitatory glutamatergic neurotransmitter receptors from cDNA libraries identified by expressing in the oocyte system (Hollmann et al. 1989; Moriyoshi et al. 1991). Eventually, oocyte expression was superseded by expression of receptors in mammalian cell lines. Compared with oocyte expression, this was less laborious, not subject to seasonal variation, and yielded higher receptor expression.

The collaboration with Brian Richards and co-workers also resulted in the cloning of the cDNA encoding the *T. marmorata* nicotinic acetylcholine receptor α subunit (25). The cDNA when translated yielded the complete α subunit primary structure. Several groups were in a race to achieve this goal, namely, those of John-Pierre Changeux in France, Shosaku Numa (ForMemRS 1986) in Japan, and Stephen Heinemann in the USA. All published nicotinic acetylcholine receptor subunit cDNA sequence data almost simultaneously. It was much disputed as to who could lay claim to be the first to report the molecular cloning not just of a nicotinic acetylcholine receptor subunit but of a neurotransmitter receptor polypeptide chain *per se*. All publications were in 1982 (Ballivet et al. 1982; Giraudat et al. 1982; Noda et al. 1982; (25)). Eric, however, always claimed to be the first! In each of these studies, once all four subunits had been cloned, notably by Shosaku Numa, expression of the respective cDNA in *Xenopus* oocytes was pivotal in showing that the identified cDNA did indeed encode functional acetylcholine-gated ion channels.
Figure 4. Responses to acetylcholine applied iontophoretically to *Xenopus* oocytes injected with mRNA isolated from the electric organ of *Torpedo marmorata*. (a) Muscarinic response to acetylcholine applied to a control oocyte (injected with H₂O). Voltage recording with membrane potential set at –64 mV. (b) Nicotinic response to acetylcholine applied to an oocyte 2 days after injection of *Torpedo* mRNA. Atropine (2.5 × 10⁻⁷ m) was used to block the muscarinic response. Voltage-clamp recording: inward current upwards. Membrane potential: –70 mV. Reproduced from (23) with permission from the Royal Society.

Eric’s group went on to clone other nicotinic acetylcholine receptor genes from invertebrates (44) and, in collaboration with David Sattelle, nematodes, e.g. (53). The deduced amino acid sequences yielded much information regarding structure/function receptor pharmacology and the evolution of nicotinic acetylcholine receptor genes.

**THE LIGAND-GATED ION CHANNEL SUPERFAMILY**

In the 1970s the existence of other neurotransmitter receptor proteins was becoming apparent by virtue of radioligand binding techniques pioneered in the late 1960s by Humphrey Rang (FRS 1980) amongst others. This method permitted a means to detect the different receptor proteins. Further, advances in electrophysiological recording methods were providing
evidence that some of these receptors, like the nicotinic acetylcholine receptors, may be fast-acting ligand-gated ion channels. One such receptor was the inhibitory $\gamma$-aminobutyric acid (GABA), later denoted the $\text{GABA}_A$ receptor. Eric applied his receptor expertise to isolate this brain receptor and subsequently to clone $\text{GABA}_A$ receptor cDNAs. This again was a significant challenge since there were no high-affinity GABA receptor ligands analogous to the snake $\alpha$-neurotoxins. Furthermore, $\text{GABA}_A$ receptors and many other brain receptors were found in the brain at low concentrations and had similar biophysical properties. Evidence was accruing to suggest that the anxiolytic benzodiazepine class of drugs, such as diazepam, mediated their therapeutic effects via interaction with $\text{GABA}_A$ receptors, but it was unclear if this was a direct effect or if it occurred via interaction with an intermediary protein. Erwin Sigel in Eric’s group exploited the fortuitous possible existence of a benzodiazepine binding site within the $\text{GABA}_A$ receptor to isolate to homogeneity, by benzodiazepine affinity chromatography, a multi-subunit protein consisting of $\alpha$ and $\beta$ subunits that was shown to have all the pharmacological properties of a $\text{GABA}_A$ receptor (26, 28). Importantly, upon reconstitution in lipid bilayers, this isolated protein was found to contain an integral $\text{GABA}_A$-gated anion channel (31).

Once the receptor had been isolated, in order to clone $\text{GABA}_A$ receptor $\alpha$ and $\beta$ subunit cDNAs, the strategy at that time was to obtain partial amino acid sequences of the subunits. This information could then be used to construct oligonucleotide probes to screen brain cDNA libraries for hybridizing cDNA clones encoding receptor subunits. It is difficult to appreciate now, in 2020, but in the mid-1980s this was not straightforward because of the amounts of receptor that could be isolated, combined with sensitivity limitations of amino acid sequencing technology. Eventually, Eric established a successful collaboration with Genentech (San Francisco), experts in the field. Many receptor preparations were shipped to the USA, often being delayed in customs. Finally, however, amino acid sequences from cyanogen bromide digests of $\alpha$ and $\beta$ subunits were obtained. This information was used in parallel in Eric’s group in Cambridge (led by Mark Darlison) and Peter Seeburg’s group in Genentech to identify and clone $\text{GABA}_A$ receptor, $\alpha$ and $\beta$ subunit cDNAs. When co-expressed in $\text{Xenopus}$ oocytes, $\alpha$ and $\beta$ subunits formed a $\text{GABA}_A$-gated anion channel with all the pharmacological traits of a $\text{GABA}_A$ receptor, including potentiation by one benzodiazepine, chlorazepate (34). This achievement changed the field.

The deduced amino acid sequences of inhibitory $\text{GABA}_A$ $\alpha$ and $\beta$ subunits revealed a shared amino acid sequence identity. Unexpectedly, despite the fact that the $\text{GABA}_A$ receptor opened a chloride anion channel through the membrane, amino acid sequence similarities were found with nicotinic acetylcholine receptors that open a cation channel. Notably a motif, the Cys–Cys loop, was evident in the extracellular domains of both receptors, leading to the recognition of a ligand-gated ion channel superfamily of fast-acting Cys-C loop neurotransmitter receptors, a landmark discovery. The cloning was published as a full article in *Nature* (becoming a citation classic; figure 5) alongside an article by Heinrich Betz’s group in Germany reporting the cloning of a cDNA encoding a subunit ($\alpha$) of inhibitory glycine neurotransmitter receptors (Grenningloh et al. 1987). It was apparent that amino acid sequence homology was shared between $\text{GABA}_A$ and glycine receptor subunits, reinforcing the idea of the ion channel superfamily (35).

There were, however, issues to be resolved. First, models of the $\text{GABA}_A$ receptor up until this point had proposed a tetrameric structure. The nicotinic acetylcholine receptor is a pentamer. In light of the homogeneity, it seemed likely that $\text{GABA}_A$ receptors should
Figure 5. The cloning of GABA_A receptor α and β subunits and the demonstration of a ligand-gated ion channel superfamily of neurotransmitter receptors (34). The image is on the 16 July 1987 front cover of Nature, in which the work was published. It shows a model of the structure of the GABA_A receptor as deduced from the primary structures of the α and β subunits. Reproduced with permission from Nature Publishing Group. (Online version in colour.)

also be pentameric, the quaternary structures being conserved between all members of the superfamily. Eric later showed this to be the case (52). Second, it became apparent that the benzodiazepine response of the cloned receptor was not robust. Peter Seeburg’s group (now relocated to Germany) resolved this by the discovery of a new class of GABA_A receptor subunit, the γ2 subunit, which endowed recombinant αβγ receptors with full benzodiazepine sensitivity (Pritchett et al. 1989).

The cloning of the GABA_A receptor α and β subunits resulted in an unforeseen finding that had profound effects for drug discovery. Early classical pharmacological studies had distinguished different types of acetylcholine receptor—muscarinic and nicotinic—both being activated by acetylcholine. Similarly, pharmacological types of adrenergic receptor were known but again based on pharmacological responses in native tissue. The dogma for brain
receptors was that for each type of neurotransmitter there should be a single neurotransmitter receptor. However, Eric and Peter Seeburg discovered further isoforms of GABA A receptor α subunits. These isoforms were encoded by separate genes but their deduced amino acid sequences shared a high identity (ca 80%) with the published α subunit. They were named the α2 and α3 subunits, with the original being designated α1. The reporting of the new subunits led to the proposal that distinct GABA A receptor subtypes exist containing either α1, α2 or α3 subunits assembled with βγ or combinations such as α1α2βγ etc. This was the first time that receptor subtypes had been distinguished purely on the basis of their molecular structures rather than by classical pharmacological means. Primarily owing to the efforts of the Seeburg group, it is known that there are 19 GABA A receptor subunits in total, resulting in extensive GABA A receptor diversity in the brain (reviewed in Olsen & Sieghart 2008). This heterogeneity, later revealed as a property common to other neurotransmitter receptor families, yields receptors with subtly distinct pharmacological properties that can be exploited for therapeutic purposes (see below).

For GABA A receptors, the cloning of receptor genes formed the foundation for elegant studies, principally from Hans Mohler’s group in Switzerland, where transgenic knock-in technology was employed to demonstrate that, in the brain, different GABA A receptor subtypes had distinct physiological functions, e.g. Rudolph et al. (1999) and Löw et al. (2000). It was shown that the anxiolytic and sedative properties of benzodiazepine were mediated by different GABA A receptor subtypes.

Eric followed up the cloning of GABA A receptor subunits by reporting the localization in the brain by in situ hybridization of GABA A receptor subunit mRNA (37); the chromosomal locations of the GABA A receptor genes (42); the first creation of stable cell lines expressing GABA A receptor subtypes (45, 50) and an investigation of their single channel properties (38, 39); conservation of the intron/exon pattern of GABA A receptor genes and the sequence and functional comparison with cloned invertebrate GABA receptors (46,47).

The availability of GABA A receptor cDNAs permitted the elucidation of the crystal structure of a recombinant homomeric GABA A receptor (Miller & Aricescu 2014) and the cryogenic–electron microscopy structure of a human α1β3γ2 recombinant GABA A receptor (Laverty et al. 2019). These major advances are crucial for understanding how they work at a molecular level. Further, since GABA is the major inhibitory neurotransmitter in the brain, dysfunction of GABAergic neurotransmission is implicated in a plethora of neurological and psychiatric disorders. Thus, knowing that GABA A receptor subtypes exist and now with the knowledge of their three-dimensional structures, computer molecular modelling is being employed for the rational design of novel, receptor subtype-selective drugs. One aim has been to find an anxiolytic drug with none of the accompanying undesirable side-effects associated with classical benzodiazepines, i.e. sedation, memory impairment, and addiction. This type of drug discovery would just not have been possible without the pioneering work carried out thirty years earlier by Eric, his research group and the research group of his collaborators, particularly Peter Seeburg.

**Other avenues and final research**

Throughout his distinguished career, Eric was at the forefront of the field with his acumen to recognize the potential of, and subsequently to embrace, emerging new technologies. This was exemplified by his move into molecular biology as described earlier. Another example
was that, in the very early days of automated DNA sequencing, he sent his PhD students in Cambridge to his former colleague at Buffalo, J. Craig Venter of human genome sequencing fame, to learn about and apply this new methodology.

With respect to neurotransmitter receptors, his research was not confined to nicotinic acetylcholine and GABA<sub>A</sub> receptors. He applied the same rationale, i.e. receptor detergent solubilization and purification, with the ultimate aim of cloning excitatory glutamate receptors and opioid G-protein-coupled receptors. Although this was not achieved by his group, nevertheless, significant contributions to the field were made. With Jeremy Henley, he developed methods for the detergent solubilization, purification and distribution of excitatory, ionotropic glutamate AMPA, kainate, and NMDA receptors from avian and fish brain (49). His studies of opiate receptors with Katy Demoliou-Mason predicted, several years before proven, that ‘All of the behaviour is interpreted in terms of a model involving association–dissociation equilibria of homologous and/or heterologous receptor subunits of an oligomeric opioid receptor structure’ ((33) and reviewed in Corbett et al. 2006). Further, Eric showed that many opioid receptor subtypes that were proposed to exist at the time, could be simply explained by the classical mu, delta and kappa receptors in different states, either coupled or uncoupled from their G-protein (48).

Eric’s last major contribution was with his collaborator from Xenopus oocyte expression days, David Brown. Many receptors are not themselves ion channels but these ‘metabotropic receptors’ activate intracellular ‘G-proteins’ that control the activity of other ion channels. David was keen to investigate his group’s data, which suggested that the P2Y2 G-protein-coupled purinoceptor in NG108-15 neuroblastoma cells may couple to two distinct currents, the potassium K<sub>s</sub>7, ‘M-current’ and voltage-gated calcium currents (Filippov et al. 1994; Filippov & Brown 1996). Eric had an interest in purinoceptors, having recently cloned the chick brain P2Y1 receptor (51). Thus the electrophysiological and cell signalling expertise of David was combined with the molecular biology and biochemical expertise of Eric. In a series of papers, in research funded mostly by the Wellcome Trust, Eric and David were able to show that the same genotypic species of P2Y2 receptor was indeed capable of inhibiting both currents. This promiscuity is at the level of the G protein, with P2Y2 being shown to have the capability to couple with either G<sub>i</sub> or G<sub>j</sub> G-proteins (54). Thus, the genotypic P2Y2 receptor behaved like a hybrid of M1 and M4 muscarinic acetylcholine G-protein-coupled receptors. Further studies on the coupling of a range of genotypically pure mammalian P2Y receptors to calcium and potassium channels in sympathetic neurons, but now using intranuclear cDNA injections, determined the principal ion-coupling paths of genotypic mammalian P2Y receptors expressed in rat sympathetic neurons (55). Finally, the role of the scaffolding protein type-2 Na<sup>+</sup>/H<sup>+</sup> exchange regulatory factor (NHERF2), a known P2Y1 interactor, in determining the downstream coupling of P2Y1 receptors was elucidated (56).

In 2008, Eric was awarded the Thudicum medal of the Biochemical Society in recognition of his contribution to molecular neuroscience. Fittingly, he was presented with the award by his long-term colleague and final collaborator, David Brown (figure 6).

**ERIC THE PERSON**

Despite a difficult childhood and early years, after his marriage to Penny, Eric enjoyed a contented, happy family life with their four children. They were married for over sixty years,
celebrating their diamond wedding anniversary in 2016. For thirty of their later years together, they lived in the converted Old School House in the village of Grantchester just outside Cambridge. Eric and Penny worked independently alongside each other, notably at Imperial College and later at the MRC Molecular Neurobiology Unit in Cambridge, where Penny was an MRC member of staff, her research focusing on muscular dystrophy. Their work together in that field contributed to the first identification of dystrophin, and eventually to the many diverse experimental therapies and clinical trials that have slowly followed.

Eric’s interests extended well beyond science. In their early years together, Eric and Penny with their colleagues at Kings, Geoff Burnstock (FRS 1986) and Lewis Wolpert (FRS 1980), were active members of the New Left Review Club, the political and arts group founded by Bernard Levin, Stuart Hall and Tony Richardson. Eric had an in-depth knowledge of modern art, classical music, and opera, being particularly fond of Wagner and the works of Shakespeare. He much enjoyed and was knowledgeable about fine wine. He had an impressive cellar and used to import wine directly from Bordeaux, listing ‘the pursuit of good claret’ as his interest in Who’s who. He loved conducting impromptu blind tastings and family members or guests would usually have to guess the wine served at lunch before it was revealed.

Colleagues who knew Eric would all concur that he was the very epitome of the absent-minded professor. At Imperial, he was affectionately referred to as the ‘white rabbit’ because of his shock of white hair and the way he would race down corridors and then seemingly disappear. His office was always full of piles of manuscript, scraps of paper containing
key information gleaned from research meetings with students and postdoctoral researchers, and reprints from which he would extract the right paper when needed, reportedly ‘like a magician pulling a white rabbit from a top hat’. In the days of Kodachrome slides, his were always prepared at the last minute, invariably held together with autoclave tape, either to keep them in one piece or sometimes (!) to conceal information. There are many anecdotes of his absent-mindedness. He reportedly turned up at an airport in the USA worrying that he had forgotten something. That ‘something’ turned out to be Penny, whom he had been supposed to pick up from home on his way from his department to the airport! Once, while racing to catch the QE2 ship from New York to London in the 1970s, he was, as often, late. He was stopped by a police officer for driving down a one-way street the wrong way. He promptly reversed at speed for several blocks. After moving to a new house in Campden Grove, Kensington, following his return to the UK, Eric was waiting at home for the decorator to arrive. The doorbell rang and the man announced that he had ‘come for the painting’. Eric was terse with him because he was running late. He gave him various instructions, which the man started to follow; but when asked to climb a ladder and start work, the man politely said, ‘I’ve come to collect the Hockney.’ The house had previously been owned by an art dealer and Eric had not noticed the visitor was not dressed for painting and decorating.

Eric’s family remember that he had a love of theatrical antics, a great sense of humour, and a penchant for puns and double entendres, often weak ones. A trademark phrase was ‘This wine is at its peak ... just like me’. His family attribute this trait to his flamboyant grandfather, Daniel Barnard, a Victorian music-hall impresario who founded Barnard’s Palace of Varieties in Chatham, Kent. Eric once famously appeared dressed as a molecule of mRNA at a Christmas party organized by his and Oliver Dolly’s group at Imperial. The fancy dress theme for the party was the twelve days of Christmas! It was difficult to see the connection.
He was also known to customize well-known songs with words that he sang himself at leaving parties for members of his research group.

Eric Barnard died on 23 May 2018. He had been suffering from bladder cancer. He was buried in the churchyard in the village of Grantchester, which had been his and Penny’s home for many years. Eric was a genuinely humble man. He had a very difficult start in life but his sister, Hilda, made the point that if he been adopted he would never have had the opportunity to go to grammar school and then university. His schooling gave him the foundation he needed for his future studies and everything he later achieved. He maintained a deep love of science throughout his life, and although he understood the scientific importance of his work he was always understated and self-deprecating about his accolades and achievements, not taking anything for granted. In the 1980s and 1990s, his MRC Unit in Cambridge was a mecca for aspiring molecular neuroscientists, many of whom have gone on to make their own significant contributions to the field. (Some can be seen in figure 7, a photograph of Unit personnel in 1988.)

Eric never lost his love for discussing experiments, including all methodology in the most absolute detail. Jean-Pierre Changeux, whose laboratory he visited for six months in 1974 to learn about the purification of the nicotinic acetylcholine receptor, remembers Eric’s sharp mind and the very friendly relationships he established with all his group, including himself. On learning of his death he said ‘Eric has been a fascinating pioneer in the chemistry of the brain and his work is fully recognized as a major milestone in the history of neuroscience. He shall illuminate our scientific memories forever’. Indeed.

HONOURS AND AWARDS

1981 Fellow of the Royal Society
1985 Ciba Medal, Biochemical Society
1998 Eli-Lilly–ECNP International Prize for Distinguished Basic Science Research in Neuropharmacology
2008 Thudicum Medal, Biochemical Society

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F. Anne Stephenson is Emeritus Professor of Molecular Neuroscience at University College London School of Pharmacy. She completed her PhD on nicotinic acetylcholine receptors at the University of Bath. She then spent a short postdoctoral period at the University of California, Riverside, with Richard W. Olsen before returning to the UK to join Eric Barnard’s group at Imperial College London. Here she became, with Eric’s support, one of the first cohort to be awarded a Royal Society University Research Fellowship in 1983. Anne worked with Eric from 1982 until 1988 before moving to the London School of Pharmacy to set up her own laboratory. Her group studied the biochemistry of inhibitory GABA$\alpha$ neurotransmitter receptors, excitatory NMDA glutamatergic neurotransmitter receptors, and mechanisms of mitochondrial transport in neurons.

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