

RICARDO MILEDI

15 September 1927 — 18 December 2017



Quilley

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Elected FRS 1970

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For nearly five decades, Ricardo Miledi was among the foremost researchers in elucidating how nerves transmit signals across synapses. Born in Chihuahua, Mexico, he qualified as a medical doctor, obtained a PhD with Arturo Rosenblueth and then, while in Canberra with John Eccles FRS, was invited by Bernard Katz FRS to join the Biophysics department at University College London, where he stayed from 1958 to 1984. Both independently and with Katz, he demonstrated that influx of calcium into the presynaptic nerve terminal is the essential trigger for the release of the neurotransmitter that carries signals across to the postsynaptic cell. He found that cutting the nerve to a frog's muscle increased the number and distribution of its muscle acetylcholine (ACh) receptors, which he purified and established as membrane proteins. Together with Katz, he introduced the technique of membrane noise analysis to determine the properties of the individual ion channels opened by ACh, providing the first functional characterization of a single receptor with integral ion channel. With Eric Barnard (FRS 1981), he pioneered a new approach facilitating the study of neurotransmitter receptors and ion channels by 'transplanting' them from brain and other tissues into large *Xenopus*

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oocyte cells by injection of messenger RNA. After moving to the University of California, Irvine, in 1984, he helped to establish the Mexican Institute for Neurobiology at Querétaro. Working in Irvine and Mexico he extended this oocyte expression technique to incorporate transplanted brain membranes, particularly from patients with epilepsy or other neurological disorders. He received many honours for his work, including the Royal Medal (1998), but was happiest working in his lab applying his extraordinary technical skills and imagination to study synaptic transmission and inspiring a generation of neuroscientists.

EARLY LIFE IN MEXICO

Ricardo Miledi was born in the city of Chihuahua in northern Mexico on 15 September 1927. His parents, Nasim and Matilde, had immigrated from Lebanon, planning ultimately to settle in the USA, to which they moved in 1955. Ricardo was the second eldest of seven siblings. As a child and at school he was noted for ‘always tinkering with things’—an early indication of the curiosity and attention to fine mechanical details that characterized Ricardo’s scientific endeavours.

From school, Ricardo first went to the Instituto Científico y Literario, Chihuahua. He wanted to study medicine, but his family lacked financial resources for higher education. An initial plan was that he and his older brother, Victor, would support each other, alternating years between earning and studying at medical school. However, Victor joined the US military and was sent to Korea, leaving Ricardo to complete his education with help from an uncle and from the local Lebanese community (supplemented perhaps by his skills at poker). At medical school, he met his future wife, Mela (Ana Mela Garces), daughter of school headmistress Esperanza Velasco de Garces and Raymundo Garces. They married in 1954, the year Ricardo graduated with an MD from the Universidad Autónoma de México. Over the succeeding 60 years, Mela was tireless in her support of his career and ambitions.

Newly graduated doctors in Mexico were required to perform social service as a component of their training. By then, Ricardo realized that he would probably not make a good clinician, as he was more interested in how the body worked in normal and disease states and how medicines might act. Fortunately, he was able to undertake a research fellowship that satisfied the social service component, working in the lab of Arturo Rosenblueth at the Instituto Nacional de Cardiología, one of the most outstanding research groups in Mexico.

At first Ricardo spent his time voraciously reading in the library. It was only when Juan García Ramos, a collaborator of Arturo Rosenblueth, noticed his obvious interest that he was allowed first to observe and then to help in their work. He taught himself how to dissect single nerve fibres and pull glass micropipettes by hand, resulting in his first publication, describing the electrical origins of ventricular fibrillation and impulse propagation in myelinated axons (1)*.

WOODS HOLE AND CANBERRA

At this stage of his life, Ricardo was a married 27-year-old whose research career had not yet taken off. A major turning point that crucially defined his future trajectory arose

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

from a serendipitous meeting with two renowned scientists, Albert Grass and Stephen Kuffler (ForMemRS 1971). They visited Rosenblueth from the Marine Biological Laboratory (MBL) in Woods Hole, MA, and were on the lookout to recruit young investigators for the nascent Grass Fellowship Program at the MBL. Intrigued by Ricardo's demonstrations of microdissection and micropipette fabrication, they suggested that he spend a summer working in Woods Hole. Ricardo jumped at the chance to immerse himself in such a rich scientific environment, and to travel outside of Mexico for the first time. Hence, he ventured north in the summer of 1955 to lecture at the MBL and to study lobster stretch receptors; along the way he made his first acquaintance with the giant synapse of the common squid, *Loligo pealii*. With this preparation began his enduring study of synaptic transmission, recognizing at first hand the importance of calcium when he inadvertently (?) neglected to add sufficient to his physiological solutions.

After returning to Mexico to complete his fellowship, Ricardo obtained a grant from the Rockefeller Foundation for an 18-month (1956/57) visit to the John Curtin School of Medical Research in Canberra, Australia. He was to work with the neurophysiologist John Eccles FRS, who later won the Nobel Prize (in 1963) alongside Alan Hodgkin FRS and Andrew Huxley FRS. An inkling of the high intensity environment of the Eccles lab came as soon they arrived in Australia. Mela remembers how two drivers met them at the airport; one to take her to their apartment, and the other to take Ricardo straight to the laboratory. Nevertheless, Eccles initially paid little attention to Ricardo—until he succeeded in setting up a complex experimental preparation that had long eluded others in the lab (figure 1). Then Eccles rapidly brought him into his own team and Ricardo began a fruitful collaboration with the Serbian neurophysiologist Kres Krnjević, studying neuromuscular transmission in isolated rat phrenic nerve/diaphragm muscle (2). During high frequency nerve stimulation, they found that after the first few successful impulses some failed to induce muscle depolarization and resulting contraction (4); adrenalin reversed the defect, probably by increasing the amount of neurotransmitter released by each nerve impulse (3).

Ricardo's time at Canberra was marked by two major life events. Mela gave birth to their son Ricardo (usually called Rico) on 17 February 1957—apparently the first Mexican to be born in Australia. The second event was a visit in 1958 by Eccles' friend and past collaborator, Bernard Katz FRS, who had recently discovered the miniature endplate potentials (MEPPs) that provided the vital clue that neurotransmitter is released from nerve terminals as individual packets ('quanta'). Towards the end of his stay in Australia, Katz offered Ricardo a position in the Department of Biophysics at University College London (UCL). Ricardo initially declined, as he had been promised a job in Mexico, but on returning to Mexico he discovered that this position had been granted to someone with better political connections. He wrote to Katz, who renewed his offer and generously agreed to provide the travel funds for his family. However, there was a crucial caveat: 'If you sign for one year, you need to repay the travel; if you sign for two years, you repay one way only, and if for three, no repayment is required' (Bregestovski *et al.* 2020). Unsurprisingly, Ricardo agreed to the three-year contract, taking up a position as honorary associate researcher in the Department of Biophysics at UCL in 1958. Thus began a quarter century of collaboration between Katz and Ricardo—surely among the most fruitful in the history of neuroscience.

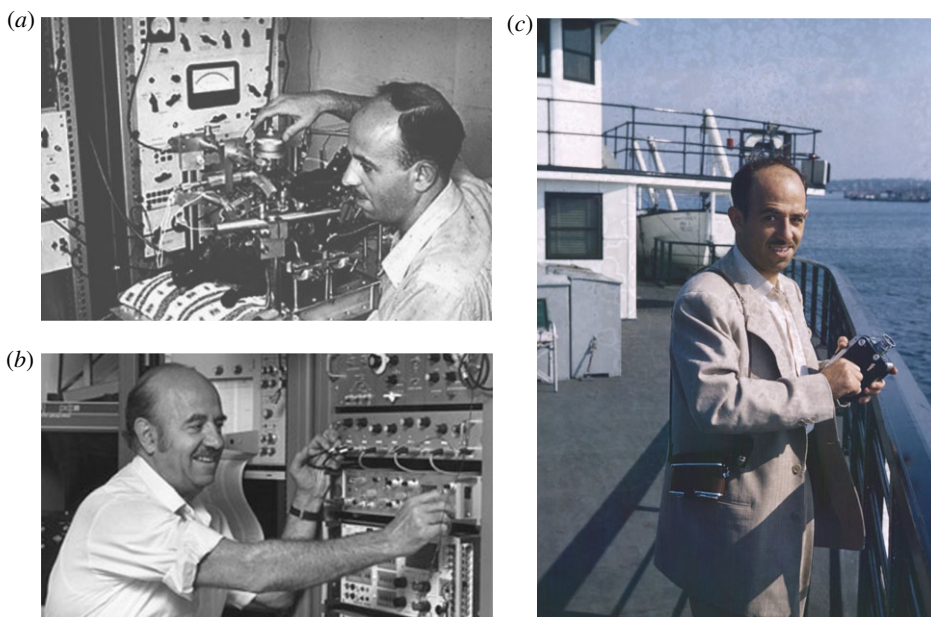


Figure 1. (a) Conducting postdoctoral work on the electrophysiology of the cat's spinal cord with John Eccles, Canberra, Australia, 1956. (b) Working on a much more upscale electrophysiology setup in the 1980s. (c) Enjoying time off at Woods Hole in the 1960s. Images (a, b) provided by Professor Francesca Grassi, courtesy of Professor P. Bregestovski and *Neuroscience* 2020. Image (c) courtesy of Rico Miledi. (Online version in colour.)

UCL BIOPHYSICS AND KATZ, 1958–1984

During Ricardo's early years in the Biophysics department, a routine was rapidly established that lasted until Katz's retirement in 1978. Two days a week, Ricardo worked with Katz; the remaining days he worked on his own projects, often in collaboration with the many postdoctoral visitors to the department. In all their publications Katz appears before Ricardo, reflecting a long-standing tradition of the Biophysics department that authors were listed alphabetically. This may seem inequitable by present day standards, but was widely recognized by others in the field, and indeed was the editorial policy of the *Journal of Physiology* until the late 1980s (Boyd 2015).

While Katz was naturally rather reserved and tended to focus on his own work, Ricardo was gregarious, convivial and extrovert. He acted as the day-to-day supervisor of many of the visitors and took an active part in social activities, including tennis on summer Sundays at the UCL sports ground at the London suburb of Shenley, and frequent meals together in the nearby restaurants of Bloomsbury and Soho. At morning coffee—an unmissable social event for visitors to Biophysics—he was keenly interactive, eloquent and amusing.

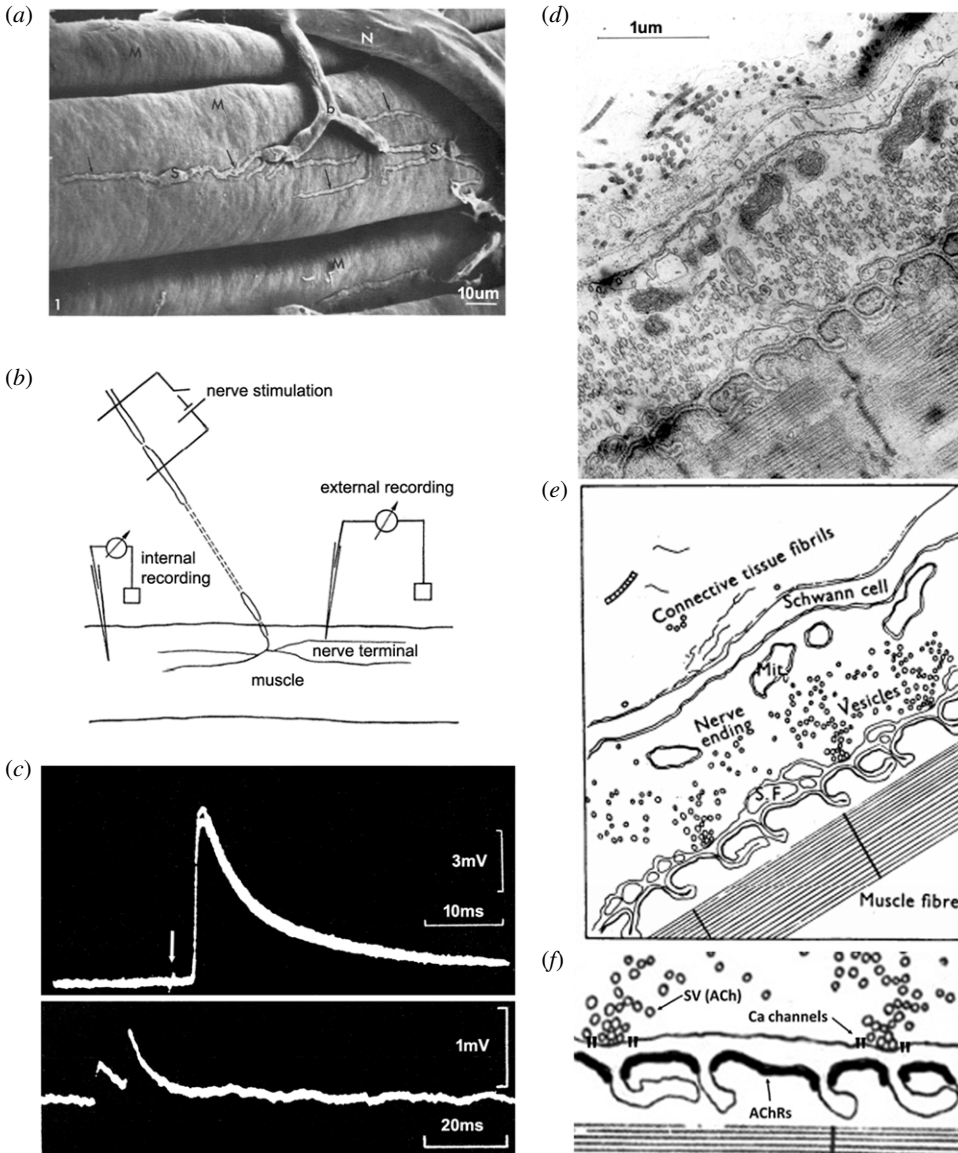


Figure 2. (Caption overleaf.)

SYNAPTIC TRANSMISSION AT THE NEUROMUSCULAR JUNCTION

Ricardo's work in the Biophysics department largely concerned how electrical signals are rapidly conveyed between nerve cells, and particularly between nerve and muscle, by a chemical intermediary (neurotransmitter). Figure 2 panels (a–c) illustrate a nerve-muscle preparation, the placement of stimulating and recording electrodes, and typical responses

Figure 2. (*Overleaf*.) The frog neuromuscular junction (NMJ), also known as the ‘motor endplate’, as studied extensively by Miledi in his early work with Katz. (a) Surface view of one NMJ, showing the Y-shaped nerve fibre terminating in several fine terminal branches that run along the surface of the muscle fibre (image from Desaki & Uehara 1981.) (b) A schematic diagram of one NMJ with electrodes for stimulating and recording electrical activity from its various cellular components (adapted from (9)). (c) Upper trace: recordings made from the muscle fibre with an internal electrode of several superimposed endplate potentials (EPPs), evoked by electrical stimulation of the nerve at the time indicated by the vertical arrow (the EPP amplitude was reduced, by addition of curare to the bathing solution, to prevent muscle excitation and contraction). Lower trace: two spontaneous ‘miniature’ EPPs (MEPPs) (adapted from (6)). (d) Electron micrograph of a small part of one NMJ. (e) Outline drawing of (d) indicating the main features; note the numerous vesicles within the nerve, clustered around discrete points on the nerve surface, and the periodic indentation of the muscle cell surface opposite those clusters ((d,e) from Birks *et al.* 1960, used with permission from John Wiley & Sons). (f) Simplified diagram of part of (e) showing the relative positions of key structural determinants of the process of neuromuscular transmission. ‘SV (ACh)’, the synaptic vesicles containing the transmitter, acetylcholine; ‘Ca channels’, Ca-selective ion channels in the nerve membrane that allow entry of Ca, which triggers ACh release; ‘AChRs’, the acetylcholine receptors, concentrated on the muscle fibre membrane surface opposite the clusters of SVs in the nerve. Note that mammalian NMJs (e.g. rat and human) are smaller and more pretzel-shaped but with highly similar molecular structures and function.

observed. Panel (d) shows the fine detail of the nerve muscle junction observed by electron microscopy, with the details illustrated diagrammatically in panels (e) and (f). Our current understanding of nerve–muscle (neuromuscular) transmission is that an electrical signal (the action potential), initiated from the brain via the spinal cord, propagates along the motor nerve into the presynaptic nerve terminal. The change in potential opens channels in the nerve membrane that allow influx of calcium ions. The resulting rise in calcium concentration inside the nerve triggers the fusion of vesicles with the presynaptic membrane, leading to the release of the neurotransmitter, in discrete packets or ‘quanta’, into the narrow gap between the nerve ending and the muscle fibre. The neurotransmitter acetylcholine (ACh) binds to large proteins (‘ACh receptors’; figure 2f) in the muscle membrane, which open their ion channels to allow positive sodium ions to enter and evoke an electrical response, the ‘endplate potential’ (EPP) (figure 2c, upper trace). This, in turn, triggers a muscle action potential, which leads to muscle contraction. Occasional, spontaneous release of the contents of individual vesicles evokes small responses, MEPPs (figure 2c, lower trace). These were discovered by Katz and provided the first clue as to the quantal nature of neurotransmitter release (reviewed by Katz 1971; Sakmann 2007). In the early 1960s, however, there were still large gaps in understanding these processes. Ricardo’s studies on the neuromuscular junction NMJ, and later on the giant synapse of the squid, underpinned subsequent understanding of synaptic transmission at all types of chemical synapses in the nervous system (Meriney & Fanselow 2019).

The main thrust of Ricardo’s work with Katz focused on two aspects of neurotransmitter release from the nerve terminal: the role of the nerve action potential and the role of calcium ions. These studies utilized the frog nerve–muscle synapse, the NMJ, chosen because the large size of the postsynaptic cell (the individual muscle fibre) and the extended morphology of the nerve ending facilitated recording and stimulation by intracellular and extracellular microelectrodes (as illustrated in figure 2b); their findings were then confirmed at the smaller rat NMJ.

Using a focal extracellular micropipette to record the electrical events from small portions of the NMJ allowed them to show that the action potential was actively propagated into these very fine terminal branches (9). By simultaneously recording the nerve action potential ‘spike’ and the resulting postsynaptic muscle fibre response, there was a minimum delay of about 0.5 ms. They concluded ‘that the synaptic interval . . . arises chiefly from a delay in the release of transmitter after the arrival of the nerve impulse’. This raised the question of the nature of the events in the nerve terminal that occur during that very brief delay (10, 11).

In previous work, Katz and his colleagues Paul Fatt (FRS 1969) and José del Castillo had shown that extracellular calcium is required for ACh release from nerve terminals (Fatt & Katz 1952; Del Castillo & Katz 1954), but it was unclear when and where calcium was involved. To pursue this question, Ricardo and Katz bathed the NMJ preparation in a calcium-free medium and pharmacologically blocked the nerve action potential. They then ejected calcium from one barrel of a double-barrelled pipette, while using the other barrel to electrically stimulate the nerve terminal (15–18). Transmitter was released only in the region of raised calcium, and only when calcium was applied at the time of electrical stimulation, leading them to conclude that calcium is concerned directly with the release of neurotransmitter, and not—as was sometimes suggested—by an effect on the nerve terminal action potential. The best way to confirm this was to see whether injecting calcium directly into the nerve terminal could induce quantal release of the transmitter, but the frog nerve terminal was too fine to test this hypothesis. During his first visit to Woods Hole in 1955, Ricardo had been introduced to the squid giant synapse preparation. This preparation permitted one or more electrodes to be inserted into the large presynaptic terminal without undue damage.

In 1964, the Grass Foundation awarded Ricardo the Alexander Forbes Lectureship for that summer session at Woods Hole. This involved giving a formal lecture, but also provided lab facilities for a research project. Ricardo and Clarke Slater (also supported by a Grass Fellowship) confirmed earlier work at Woods Hole (Bullock & Hagiwara 1957; Hagiwara & Tasaki 1958; Takeuchi & Takeuchi 1962), showing that, as in the frog NMJ, transmission in the giant synapse in calcium-free medium could be restored locally by application of calcium from a pipette (12). Ricardo recalled that ‘I thought, if all I need is the calcium inside, that should release neurotransmitter . . . But when I injected calcium, I didn’t see any response’ (Jeng 2002). As they had not seen any miniature synaptic potentials at the giant synapse, however, it appeared that methodological limitations were impairing their ability to detect release following calcium injection.

Lacking funds to visit Woods Hole again, and at the suggestion of J. Z. Young FRS, who had originally described the giant synapse, Ricardo began summer work at the Stazione Zoologica in Naples. Andrew Packard, the director of the Stazione’s zoology department, and his successor, Rainer Martin, had an arrangement with local fishermen to provide fresh, hand-caught squid each day. On his first visit, Ricardo was accompanied by Katz, and they set to work measuring the relation between presynaptic membrane potential and transmitter release (13). Ricardo used particularly small squid with smaller axons, and was then able to observe spontaneous miniature depolarizing potentials (14, 19), similar to the MEPPS seen at vertebrate NMJs. With this preparation, injection of calcium directly into the nerve terminals evoked release of transmitter, even when the action potential was blocked and possible entry of extracellular calcium was prevented using a calcium-free medium (27).

Taken together, Ricardo’s experiments on the NMJ and squid giant synapse provided the unequivocal evidence to establish the calcium hypothesis of neurotransmitter release, and to

demonstrate that the site of calcium action is indeed inside the nerve terminal. This work contributed to Ricardo's election to the Royal Society (1970) and prompted many further studies, including determination of the molecular machinery by which intracellular calcium ions stimulate vesicle fusion by Thomas Südhof (ForMRS 2017, Nobel Laureate 2013) (Südhof 2013).

In the early 1970s, the administration and organization of the Stazione Zoologica was in transition, complicating Ricardo's visits. Katz had been awarded the Nobel Prize in 1970, and with the added recognition came responsibilities outside the laboratory, leaving Ricardo to assume a greater role in the university, and in teaching and training. Moreover, Katz and Ricardo's seminal work on 'ACh noise' analysis was moving to the forefront, and Ricardo's scientific focus was shifting from the presynaptic to the postsynaptic muscle membrane. These factors contributed to the eventual end of Ricardo's era in Naples. He continued his annual trips to the Stazione Zoologica until 1973, returning for a final time in 1977 with Ian Parker (FRS 2008) and colleagues to conduct experiments on the giant synapse using a metallochromic optical indicator to monitor calcium influx across the presynaptic (40) and postsynaptic membranes (54).

NOISE ANALYSIS: ESTIMATING SINGLE-CHANNEL PROPERTIES

By the late 1960s it was recognized that a receptor for ACh in the postsynaptic membrane opened an integral ion channel, allowing sodium ions to enter down their concentration gradient and depolarize the postsynaptic cell. However, measurement of the tiny current flowing through an individual channel, and the resulting depolarization, appeared far below the resolution of electrophysiological techniques available at the time. A way to circumvent this limitation came from the astute observation by Katz and Ricardo that the increase in electrical 'noise' that accompanies the depolarization evoked by ACh might convey useful information. By proposing that this arises from stochastic fluctuations in the numbers of open channels (elementary 'blips') caused by random binding of ACh molecules to ACh receptors, they introduced to neuroscience the technique of stationary noise analysis, allowing the electrophysiological characteristics of individual ion channels to be derived from macroscopic whole-cell responses (22, 25).

Their first analyses were carried out by measuring fluctuations from tracings on paper, but were soon automated by Link8 computers installed by George Dawson in the adjacent Physiology department. Recordings were captured by a tape recorder in the lab and transmitted to the computer through cables strung down a long hallway and a flight of stairs. Data were initially obtained by intracellular voltage recording, where the elementary signals were filtered and distorted by the slow electrical time constant of the muscle fibre. To estimate more accurately the mean duration of the brief elementary blips, they then recorded the transmembrane current with a focal extracellular pipette—a technically easier method than voltage-clamping the muscle fibres.

Their studies provided the first functional characterization of single biological molecules in living cells. Major findings included the first estimate of the conductance and gating kinetics of single ACh receptor channels, measurement of the number of channels activated by a single quantum of transmitter at the NMJ and the discovery that the mean open-time of these channels differs when activated by different agonists (22, 25, 28). Subsequently, Ricardo,

together with Chuck Anderson and Stuart Cull-Candy (FRS 2002), applied noise analysis of voltage-clamped recordings to study the excitatory glutamate receptors and inhibitory gamma-aminobutyric acid (GABA) receptors which occur widely at vertebrate and invertebrate synapses (33, 34).

These findings paved the way for the subsequent development of the patch-clamp technique for direct resolution of single-channel currents by Bert Sakmann (ForMemRS 1994) and Erwin Neher (ForMemRS 1994) (Neher & Sakmann 1976), who were awarded the Nobel Prize in 1992.

DENERVATION, ACETYLCHOLINE RECEPTORS AND MYASTHENIA GRAVIS

Ricardo's early work at UCL concerned the sensitivity of rat skeletal muscles to ACh, and the changes that occurred after cutting the nerve input (reviewed in Eusebi 2007). He applied ACh from a micropipette onto the surface of rat muscles and recorded the resulting depolarization of the muscle membrane. In normal, mature muscle, this ACh sensitivity is restricted to the NMJ (where the ACh receptors are concentrated as illustrated in figure 2f), but following denervation the entire muscle surface becomes sensitive to ACh (5); this was also the case during fetal and early postnatal life before innervation (8). During this time, Ricardo made the surprising discovery that, a few days after cutting the nerve, MEPP-like potentials reappeared (7). Subsequent studies with Clarke Slater, using electron microscopy (a newly-acquired skill), showed that these MEPPs arose from the glial Schwann cells which surround and support nerve axons rather than from any 'surviving' nerve terminals (20, 21, 26, 29), establishing the ability of non-neural cells to release transmitter in a quantal fashion.

By the 1970s, and following the discovery of membrane noise, Ricardo focused more on the ACh receptors. Although these were widely assumed to be membrane proteins, how could one identify a molecule that functions only when it is integrated into the lipid membrane of a muscle fibre? Ricardo and colleagues used ^{131}I - α -bungarotoxin, a snake venom toxin that binds ACh receptors irreversibly (Chang & Lee 1963), to identify these receptors in solubilized *Torpedo* electric tissue (23), and show that the distribution and time course of expression of ACh receptors in adult and denervated frog muscles (24) was similar to the ACh sensitivity described earlier (5, 8).

These developments helped to establish the ACh receptor as a distinct molecule, but it needed to be purified in order to determine its protein nature. Ricardo, together with David Green, used affinity chromatography (Cuatrecasas *et al.* 1968) to extract the α -bungarotoxin-binding material as relatively pure ACh receptor (AChR) protein (23, 31) — a first for a membrane protein. Michael Raftery (FRS 1986) subsequently determined the amino acid sequences of the N-termini of the different subunits that make up the AChR (Conti-Tronconi *et al.* 1982) and cloning of the cDNAs for each AChR subunit then followed (Noda *et al.* 1982; Sumikawa *et al.* 1982)—another first for membrane proteins.

With his medical background, Ricardo was well aware of the classic neuromuscular transmission disorder myasthenia gravis (MG), in which patients become weak and fatigued. A group in Lund, Sweden (Elmqvist *et al.* 1964), had reported reduced MEPP amplitudes at the NMJs of patients, but they could not at that time measure quantitatively the putative ACh receptors. Ricardo wanted to use ^{125}I - α -bungarotoxin to do this. His distinguished neurophysiologist friend, Tom Sears, suggested he contact John Newsom-Davis (FRS 1992), a

neurologist looking after patients with severe myasthenia (Vincent 2019). Ricardo's studies on the NMJs of MG patients confirmed the reduction in MEPP amplitudes and, as first reported by Fambrough *et al.* (1973), a reduction of ^{125}I - α -bungarotoxin binding sites, strongly supporting a postsynaptic defect in the numbers of ACh receptors (36).

Ricardo also wanted to raise antibodies to the purified AChR for further studies on the function, structure and localization of the AChR. Rabbits injected with purified *Torpedo* AChR developed strong antibody responses and, shortly after developing these antibodies, the animals became overtly weak (30). Similar observations were made at the Salk Institute, suggesting that this could provide a useful model for MG (Patrick & Lindstrom 1973). Ricardo found that the diaphragm muscles from the immunized rabbits displayed smaller MEPPs and reduced numbers of ACh receptors, resembling the changes at the NMJs of MG patients (30, 31).

This became a fast-moving field when Jon Lindstrom, turning down an invitation to work with Ricardo at UCL, demonstrated human autoantibodies binding to the AChR in around 85% of MG patients (Lindstrom *et al.* 1976). Ricardo generously let Newsom-Davis take over the clinical research, which had begun to involve patients with genetic as well as autoimmune forms of myasthenia (Vincent *et al.* 1981). At the Royal Free Hospital, Newsom-Davis quickly confirmed the presence of the antibodies and established that patients made a remarkable recovery after therapeutic plasma exchange, which lowered the levels of antibodies (Newsom-Davis *et al.* 1978).

These studies clearly demonstrated that the numbers of AChRs were reduced in MG; but was the function of the remaining AChRs altered by the binding of antibodies? With Cull-Candy, Alain Trautmann and Osvaldo Uchitel, Ricardo undertook the first voltage-clamp of human endplates to demonstrate by noise analysis that the properties of the ACh-activated channels are virtually unchanged in MG and in immunized rats, indicating that the loss of sensitivity to ACh indeed results mainly from loss of functional ACh receptors; furthermore, the change was accompanied by enhanced presynaptic release (35, 38, 39, 41).

To look at this presynaptic change in MG, Ricardo recruited Peter Molenaar to directly measure release of ACh from muscle using gas chromatography/mass spectrometry (42, 46). They found the amount of ACh released from MG muscle by nerve stimulation was approximately double that in healthy muscles; surprisingly, the same increase was seen when rat muscle receptors were blocked by incubation in α -bungarotoxin for as little as one hour (37). Thus, compensatory presynaptic changes occurred when postsynaptic function was impaired, either acutely or longer-term.

NATIVE MEMBRANE CURRENTS IN *Xenopus* OOCYTES

In 1977 Ricardo published a short letter to *Nature* (32) that marked a change in direction, describing an experimental preparation that was to define the remaining almost four decades of his scientific career. Musing on the early appearance of sensitivity to ACh during differentiation of muscle fibres, he wondered when ACh receptors first appeared. His experiments examining muscles at earlier and earlier stages during development became increasingly difficult, until he decided to approach the problem from the other end, asking: Are oocytes (immature egg cells) sensitive to ACh? He commented that 'At that time I did not fully anticipate all the consequences that would follow from such a simple question' (32).

Voltage-clamp recordings from *Xenopus laevis* oocytes indeed showed that these cells responded to ACh, but with features very different from the responses in muscle. In contrast with the fast, smooth currents carried primarily by Na^+/K^+ currents in muscle, currents in oocytes began after a long latency, displayed characteristic oscillatory fluctuations, and were mediated primarily by Cl^- ions. Ricardo and his co-authors (32) speculated that ‘the opening of the channel is . . . triggered indirectly through the production of a substance within the cell’. A subsequent paper (43) showed that muscarinic ACh receptors, rather than the nicotinic ACh receptors at the NMJ, underlie the ACh-evoked current and confirmed chloride ions as the charge carrier.

In addition to agonist-activated currents, Ricardo discovered that *Xenopus* oocytes also exhibit voltage-activated currents. In response to voltage-clamped depolarizing pulses, he observed a transient outward current carried by chloride ions and dependent on extracellular calcium (44). Further experiments (49) showed that the chloride current could be directly evoked by intracellular injection of calcium and blocked following intracellular injection of the calcium chelator EGTA. This confirmed the notion that calcium entering through voltage-gated channels opened chloride-permeable membrane channels. This was the first description of a chloride channel activated by cytosolic calcium, a feature subsequently identified in many cell types (Hartzell *et al.* 2005). Further, these results suggested a mechanism underlying the oscillatory responses mediated by muscarinic and other metabotropic receptors, in which cytosolic calcium elevations evoke chloride currents. The remaining link between cell surface receptors and cytosolic calcium was revealed when Michael Berridge (FRS 1984), Robin Irvine (FRS 1993) and collaborators (Streb *et al.* 1983) identified inositol trisphosphate (IP_3) as an intracellular messenger that is generated by hydrolysis of membrane phospholipid following receptor activation and liberates calcium ions from internal stores. Indeed, injection of IP_3 into oocytes mimicked the muscarinic response (Oron *et al.* 1985), and Ricardo and Parker further showed the oscillatory chloride current is accompanied by increases in cytosolic calcium (55). These pioneering findings led to the widespread use of the *Xenopus* oocyte as a model system for studies of calcium signalling (Lin-Moshier & Marchant 2013), including the discovery of local calcium ‘puffs’ as ubiquitous building blocks of IP_3 -mediated signalling (Parker & Yao 1991).

THE OOCYTE mRNA EXPRESSION SYSTEM

Beginning in the 1980s, Ricardo helped to pioneer a new approach to study the brain by ‘transplanting’ receptors and channels from small and relatively inaccessible neurons into large oocyte cells, where they could be more easily studied. The basic method involves injecting messenger ribonucleic acid (mRNA) from brain into the oocytes, where it is translated to form receptor and channel proteins that are functional in the plasma membrane (56, 58) (figure 3).

The utility of *Xenopus* oocytes (immature frog eggs) for translating proteins encoded by microinjected mRNA was pioneered by Sir John Gurdon (FRS 1971) (Gurdon *et al.* 1971), who demonstrated translation of haemoglobin and postulated that all kinds of eukaryotic mRNA may be capable of translation in this system. This was applied to the nervous system by Eric Barnard (FRS 1981; see Stephenson 2020) who, together with Katumi Sumikawa and colleagues, expressed nicotinic AChR from mRNA obtained from the electric organ of

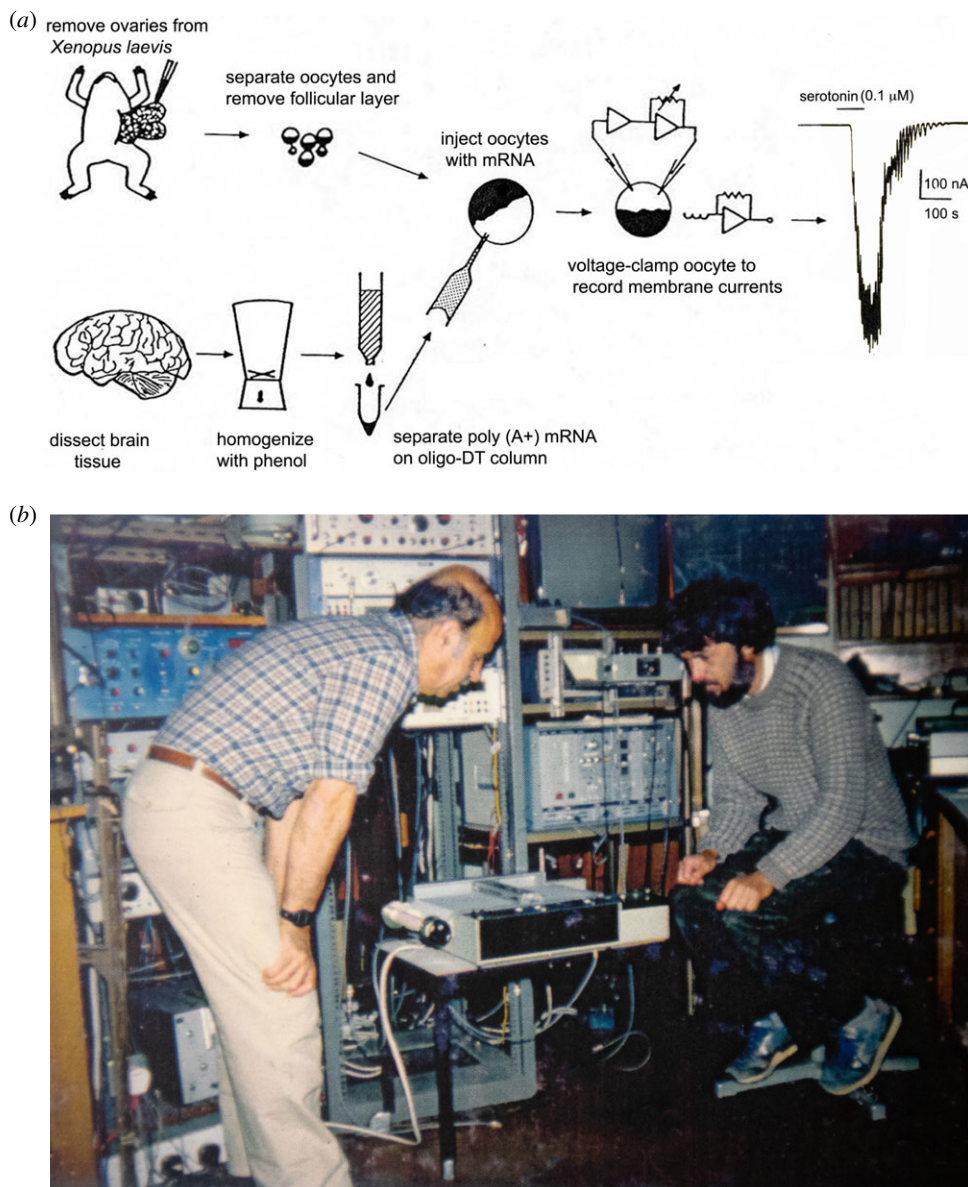


Figure 3. Development of the oocyte 'transplantation' system. (a) Poly(A)⁺-mRNA was obtained by chloroform-phenol extraction and enriched by oligo-dT chromatography for injection into oocytes isolated from ovaries of *Xenopus laevis*. Recordings of membrane current mediated by expressed receptors and channels were obtained from oocytes a few days after injection using a two-electrode voltage-clamp. The trace shows a typical 'oscillatory' chloride current evoked by application of serotonin to an oocyte injected with rat brain mRNA. (b) Ricardo and Ian Parker eagerly watching the chart recorder for evidence of successful expression of neurotransmitter receptors. Photograph taken in the Biophysics department of UCL in 1983, and used courtesy of Ian Parker. (Online version in colour.)

the electric ray *Torpedo marmorata* (Sumikawa *et al.* 1981). In those first experiments, the nicotinic ACh receptors were detected with the radiolabelled snake α -neurotoxin, leaving open the question of whether the receptors were functional in the oocyte membrane. Partnering with Ricardo for electrophysiological studies, Barnard and Sumikawa demonstrated the synthesis and incorporation of functional receptors that opened ion channels like those of native nicotinic receptors (45). This was the most direct way, at that time, to demonstrate that the ACh-sensitive ion channel was integral to the AChR protein.

Using this astonishingly simple procedure, Ricardo was soon able to induce the oocyte to acquire most of the known neurotransmitter receptors and voltage-operated channels using mRNAs from neuronal tissue of non-mammalian vertebrates and mammals (57). By that time, it was clear that synaptic transmission between neurons in the brain was complex, with a large range of receptors and ion channel proteins involved. In a rapid-fire series of papers published from UCL, with brain mRNAs prepared initially by Sumikawa and then by Cameron Gundersen, Ricardo demonstrated functional oocyte expression of receptors and channels including inhibitory type A GABA (47) and glycine receptors (53); serotonin receptors (48); voltage-gated sodium and potassium channels (50); noradrenalin and dopamine receptors (51); and excitatory kainate-type glutamate receptors (52). Remarkably, this 'transplantation' approach remained effective even for post-mortem human brain tissue (50).

FROM UCL TO UCI

Bernard Katz retired in 1978, leaving the UCL Biophysics department in Ricardo's hands. Ricardo grew increasingly disenchanted with this role—an administrative responsibility made particularly onerous by the anomalous status of a highly active research department that did little undergraduate teaching. Moreover, Mela had become unhappy with life in England and encouraged him to consider moving. Thus, although Ricardo had turned down attractive US offers at earlier stages in his career, he was now pleased to accept the offer of a position as distinguished professor in the Department of Psychobiology (now Neurobiology and Behavior) at the University of California, Irvine (UCI). The campus was established only in 1964, and the Psychobiology department was the first institution in the world dedicated to neuroscience. Following the completion of a new building for the Center for Neurobiology of Learning and Memory, ample space became available in the main biological sciences building (Steinhaus Hall), which Ricardo was able to remodel to his requirements. He was allocated two assistant professor positions, which allowed him to take with him Ian Parker and Katumi Sumikawa. Ricardo moved to UCI in 1984, commenting that he 'was leaving from a place where history was made for one where history was being made'. UCI was then embarking on an experiment to construct on-campus housing for faculty and staff. Ricardo, Mela and Rico moved into their new home in University Hills, a 20-minute walk from the lab, set high on a hillside with expansive views from the garden across an ecological preserve of coastal sage scrub.

Ricardo rapidly resumed his work on the oocyte expression system at UCI. One aspect derived from the observations that the receptors for some neurotransmitters, serotonin, glutamate and noradrenalin, as well as muscarinic receptors, also evoked characteristic oscillatory chloride currents. Examining interactions between these agonists led to the conclusion that, although each acts on a distinct receptor, they all 'link-in' to a common pathway wherein IP₃ signalling and oscillatory calcium liberation lead to the opening of

chloride channels (58). Ricardo's observations that glutamate activates this pathway (52) illuminated a new type of glutamate receptor. Until then, these receptors were thought only to activate an intrinsic ion channel. Ricardo's observations added to the growing evidence for metabotropic glutamate receptors, which link to ion channels via intracellular pathways to modulate other neuronal functions (Fong *et al.* 1988).

In the 1980s and early 1990s, the systems available for expressing membrane proteins were not well developed. Hence, the ability to identify and study previously unknown membrane proteins by injecting mRNA opened new possibilities, representing a paradigm shift in studies on the pharmacological and biophysical properties of receptors and ion channels, as well as a method for expression cloning of receptors (Romero *et al.* 1998) and for examining mutant receptors created by genetic engineering. Facilitating these studies, the popularity of the oocyte expression system drove commercial development of electrophysiology equipment for voltage- and patch-clamp work and high-throughput screening of oocytes in multi-well plates. Although expression of receptors in mammalian cell lines began to supersede the oocyte expression system around the turn of the century, it has remained in popular use; a PubMed search for '*Xenopus* oocyte expression' retrieved nearly 4000 publications from 2010 to 2020.

By using the oocytes to express receptors encoded by mRNA from the retina, Ricardo discovered a new class of inhibitory GABA receptor (GABA_C), with kinetic and pharmacological properties qualitatively different from those previously known. He initially identified this novel type of receptor by its resistance to block by the commonly used GABA antagonist bicuculline (59). Subsequently, he went on to clone the receptor (62), and to develop its first selective antagonist (61), with potential utility for treatment of central nervous system disorders.

QUERÉTARO 1995–2017

During his last 20 years, Ricardo played a major role in building up the Institute of Neurobiology (INB; formerly the Center of Neurobiology) of the National Autonomous University of Mexico (UNAM), which had first been established in 1993. The sudden appearance of a charismatic and strong scientific gravitational force attracted national and international visibility to the young INB and secured funds for the construction of modern facilities in the city of Querétaro. The INB grew as a consolidated institute of UNAM, comprising 50 researchers working in diverse areas of neuroscience, from the molecular and cellular to integrative neurosciences. Ricardo supported recently-graduated PhD students of the INB to obtain postdoctoral positions under the Pew Latin American fellowship programme and, with the support of the Grass Foundation and the Society for Neuroscience, instituted an international training programme in Latin America. Originally named the Miledi Neuroscience Training Program, this one-month workshop enables students from Latin American countries to explore the frontiers of the neurosciences, joining with international faculty, many of whom are outstanding trainees of Ricardo.

Ricardo led an initial group at the INB, which included three of his collaborators from UCI. Rogelio Arellano, investigating the ion currents and receptors of frog oocytes founded the cellular neurophysiology laboratory, devoted to understanding the process of myelination; Jesus Garcia, working on the effect of antidepressant molecules and metal ions on nicotinic

receptors; and Ataulfo Martínez, continuing the study of GABA_C receptors. Among Ricardo's own research accomplishments while working at the INB, he elucidated the physiology of muscarinic- and ATP-evoked electrical signals in *Xenopus* and mammalian oocyte follicles (71), and extended his studies of GABA_C receptors (69), including determination of their structural domains (63).

When in Querétaro, Ricardo's academic 'family' from around the world would get together for scientific seminars, laboratory meetings and social gatherings in Casa La Rana, the beautiful house that Ricardo and Mela built in traditional Mexican style, decorated with frogs on facades and doors, and ornaments brought by his many friends and colleagues. The inspiration and friendship he provided is very evident in the *Neuroscience* volume devoted to his outstanding work and edited by Ataulfo Martínez-Torres, John Heuser and Rafael Gutierrez (Martínez-Torres *et al.* 2020) (figure 4).

TRANSPLANTATION OF HUMAN BRAIN AND MUSCLE MEMBRANES

In a final stage of his career, working at UCI and in Mexico, Ricardo developed another new approach for 'transplanting' ion channels and receptors, incorporating membrane fragments rather than mRNA into *Xenopus* oocytes (65). He initially demonstrated that injection of membrane vesicles from the *Torpedo* electroplaque led to the rapid appearance of functional ACh receptors and chloride channels, indicating that the injected membranes had fused with the oocyte plasma membrane (60).

Among many subsequent applications, this approach allowed Ricardo and colleagues to study the ACh receptors in muscles from patients with motor neuron disease (MND). Although MND was known to be due to motor neuron degeneration, it had never been clear whether the muscle is directly or indirectly affected. Their results (70, 73) showed that the affinity of the ACh receptor for ACh is decreased in amyotrophic lateral sclerosis (ALS) and that riluzole, a drug with modest beneficial clinical effect, reduces ACh receptor function. The revolutionary approach of membrane transplantation also proved particularly useful for the study of receptors and ion channels derived from postoperative human brain tissue; receptors and channels could be 'resuscitated' even from tissues kept frozen for many years (68). For example, in collaboration with the group of his great friend, Fabrizio Eusebi, at the Sapienza University in Rome, and using temporal lobe tissue from patients with intractable epilepsy (who undergo surgery to excise the seizure focus), he was able to study GABA-, kainate- and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors. He showed that the GABA receptor function declined more rapidly than in brain membranes from unaffected subjects, consistent with a defect in inhibitory activity (64, 66). Using similar approaches, Ricardo was able to study, for the first time, the properties of receptors in their native membranes with relevance to autism (67) and other nervous system disorders, such as Alzheimer's disease (72).

RICARDO IN HIS LAB

Ricardo was above all a consummate and dedicated experimentalist, often working in the lab seven days a week and continuing well into his eighties. He continued this regime even when health issues confined him to morning experiments, with Mela driving down to bring

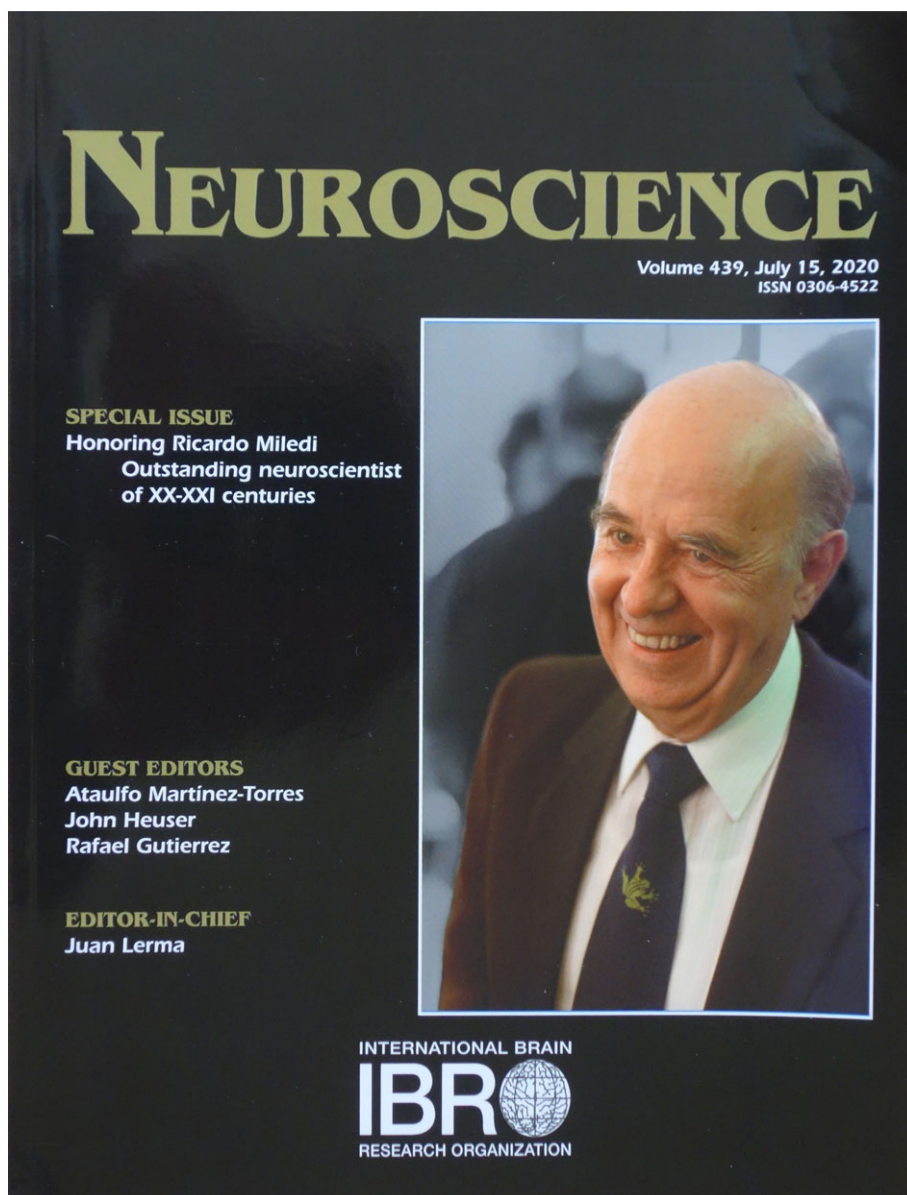


Figure 4. The *Neuroscience* volume in his honour, edited by Ataulfo Martínez-Torres, John Heuser and Rafael Gutiérrez and published in 2020. Reproduced from the cover of *Neuroscience* volume 439, © 2021 International Brain Research Organization, with permission from Elsevier. (Online version in colour.)

him home for lunch. Indeed, most of his key experimental findings were obtained with his own hands. For the most part, Ricardo took charge of the critical positioning of the electrodes while Katz or other colleagues controlled the electronics and recording. Working in the lab

with Ricardo was a career highlight for many researchers. Although perhaps nominally only serving as scribes, he fully engaged even the most junior students with the rationale behind the experiment, asking their opinions and what he should do next in the experiment.

Throughout his career Ricardo utilized equipment that was technically simple, even archaic, but with which he was intimately familiar and which he applied with great dexterity and ingenuity (see figure 1). Electrodes were positioned with ‘grease plate’ micromanipulators and viewed through a compound microscope with the resolution to identify individual neuromuscular terminals. Ricardo had functional vision in only one eye, making his manual dissecting skills all the more remarkable. Far from being put off by this, he put it to advantage by having different magnification eyepieces, allowing him to readily switch between two magnifications—and to tease visitors to his lab by asking them to look through the microscope without informing them beforehand!

The series of ground-breaking studies on quantal neurotransmitter release reported from 1965 to 1967 hinged on the potent combination of increased visibility through the compound microscope and his ability to place pipettes in precise locations along the motor nerve terminal. Ricardo prepared his own electrodes using a home-built ‘wooden’ puller and, to enable them to fit under the microscope objective lens, he elegantly bent the tips using a De Fontbrune microforge. He continued to use valve (vacuum tube) cathode-follower microelectrode amplifiers for many years into the transistor age, until the unavailability of the requisite 90 V batteries necessitated an upgrade. During most of Ricardo’s time in London and Naples, recordings were captured on 35 mm film from an oscilloscope screen using a camera with a manual wind-on lever and a shutter pressed precisely long enough to give a desired exposure time. The camera was not shielded, so experiments were done with the lab lit by only a dim red bulb, imparting a sense of mystery to anyone passing by the partially open door. Only after a chart recorder was acquired to record the slower responses from *Xenopus* oocytes was it possible to work under normal illumination (figure 3).

Ricardo had a remarkable ability to spot the ‘hidden gold’ that would pass most scientists unnoticed. As his interests widened he appreciated the possibilities of the new molecular biology of receptors and ion channels, and of *Xenopus* oocytes to examine their properties. He had a particular talent for taking a novel concept, such as the transplantation of human membranes from epileptic brain tissue, and with imagination and creativity devise a relatively simple experiment to test the validity of the concept. He then applied the approach to examine multiple different phenomena, such as the characterization of GABA receptor function in human epilepsy, that could not otherwise be easily investigated.

Ricardo sought at all times to stay out of the media spotlight, despite the countless awards and recognitions he received. He was a true exemplar of discretion, honesty and tenacity. He strove to facilitate the creation of multicultural environments in his laboratory (at times the researchers in his lab hailed from 14 different countries) and was always committed to the most disadvantaged, even opening the doors to people at risk of social exclusion, to show them other ways. He approached research with enthusiasm, tolerance and humour, training and inspiring many generations of students, postdocs and co-workers who are now leaders in academia and industry the world over. In 2002 and 2007 (the years of Ricardo’s seventy-fifth and eightieth birthdays), many of his previous students and visiting fellows met for three days of wonderful science and reminiscences in Rome, organized by Francesca Grassi and Fabrizio Eusebi, with the second opened by Nobel Laureate Rita Levi-Montalcini ForMemRS (figure 5). His legacy is remarkable in its breadth and depth.



Figure 5. (a) Participants at Ricardo's seventy-fifth birthday meeting in Rome 2002. (b) Ricardo with Nobel Laureate Rita Levi-Montalcini at his eightieth birthday celebration in 2007. (c) With Mela at the conference dinner. (d) Ricardo with Kres Krnjević. Photographs provided by the authors, with thanks to Francesca Grassi for organizing the meetings. (Online version in colour.)

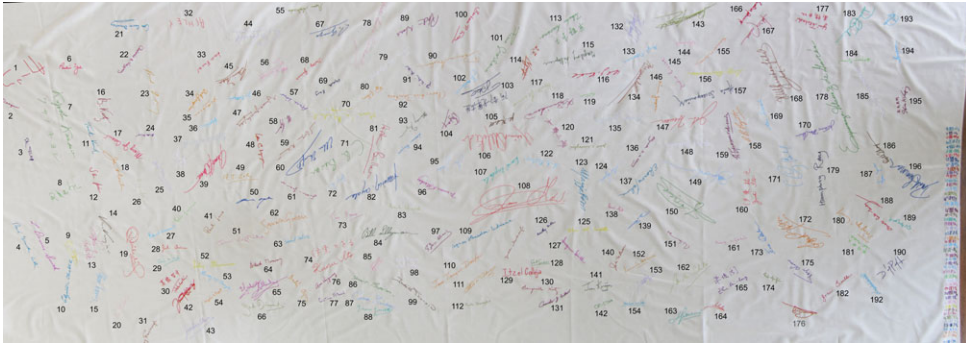


Figure 6. The famous tablecloth with signatures (numbered) of many of those entertained by Mela and Ricardo. The different colours indicate the years of the dinner from 1973 to 2011 (see right margin). Names of particular relevance in their friendships and achievements are: 13 Bernard Katz; 14 Torsten Wiesel (ForMemRS 1982); 17b Kres Krnjević; 18 John Eccles; 20 Ricardo Miledi; 22 Ian Parker; 23 Doug Fambrough; 32 Tomoyuki Takahashi; 35 Fabrizio Eusebi; 51 Hersch Gershenfeld; 143 Semir Zeki (FRS 1990); 153 John Heuser; 156 Jacques Stinnakre; 162 Mela Miledi; 66 Piotr Bregestovski; 76 Humphrey Rang (FRS 1980); 178 Terje Lømo; 189 Stuart Cull-Candy. A list of names of all identified individuals is provided as supplementary material with the online version of this article. Sincere thanks to Rico and Mela Miledi for all the work involved in creating this image and identifying the signatures. (Online version in colour.)

FAMILY LIFE

Ricardo and Mela's son, Rico, started playing with computers in his teens and became an essential part of the team, with his programming skills applied successively to the many developments in computing and programs for use in the electrophysiology lab over the next decades; he moved with them to California, where, until recently, he worked in IT for the School of Biological Sciences at UCI. Ricardo's greatest support, however, was undoubtedly his beloved and inseparable Mela, whom he adored and whom he recognized as 'the hidden side of his success'. It was Mela who, in coordination with Ricardo, was in charge of 'taking care' of their disciples, even in the smallest details. Mela and Ricardo would host magnificent dinners for colleagues from the lab and visiting researchers, plentifully supplied with wine, often beginning with caviar and blinis, and ending with Mela's classic tarte tatin. Guests were seated around an enormous table with a white tablecloth, on which they were invited to sign their names. Mela then embroidered each name, preserving a unique record of almost 200 signatures, including many of the most distinguished neuroscientists of Ricardo's era (figure 6).

AWARDS

1955	Grass Fellow, Woods Hole, Massachusetts
1956–1958	Rockefeller Foundation Fellow, John Curtin School of Medical Research Canberra, Australia
1964	Forbes Lecturer, Woods Hole, Massachusetts

1970	Fellow, the Royal Society, London
1972	Member, the Royal Institution
1983	Fellow, the Third World Academy of Sciences
1986	Fellow, the American Academy of Arts and Sciences
1987	Luigi Galvani Award
1988	King Faisal Foundation International Prize for Science
1988	Honorary Member, Hungarian Academy of Sciences
1989	Member, the National Academy of Sciences USA
1991	McKnight Research Award
1991	Member, the Mexican Academy of Sciences
1992	Doctor Honoris Causa, Universidad del Pais Vasco, Leioa, Spain
1992	Member, the European Academy of Arts, Sciences and Humanities
1995	Catedra Miguel Aleman, Mexico
1995	Member, the Mexican Academy of Medicine
1995	Fellow, the American Association for the Advancement of Science
1998	Royal Medal of the Royal Society, 'In recognition of his many important discoveries in cellular and molecular physiology which have greatly advanced our knowledge of synaptic transmission in the nervous system and of long term effects of trophic interaction between neurones and effector cells'
1999	Principe de Asturias Prize
2000	University of California, Irvine Medal
2000	Doctor Honoris Causa, Universita di Trieste, Italy
2000	Doctor Honoris Causa, Universidad Autonoma de Chihuahua, Mexico
2002	Doctor Honoris Causa, Universidad Autonoma de Querétaro
2010	Society for Neuroscience Ralph W. Gerard Prize

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AUTHOR PROFILES

Ian Parker FRS

Ian Parker is currently a distinguished professor in the Department of Neurobiology and Behavior at the University of California, Irvine. While a graduate student in the Physiology department at University College London (UCL), he was enlisted for his programming skills by Ricardo Miledi to write software for noise analysis of postsynaptic currents. That led to an invitation to join Ricardo's lab as a research assistant, where he worked between 1975 and 1984 on projects including the roles of calcium in synaptic transmission and excitation–contraction coupling in muscle, and for the last two years helped develop the oocyte transplantation technique for study of ion channels and receptors. Accompanying Ricardo, he moved in 1984 to UCI, where his main focus is on elucidating the spatiotemporal patterning of cellular calcium signalling—most notably discovering the local calcium 'puffs' that constitute a basic building block of calcium signals. Along the way he developed novel imaging techniques, including 'optical patch-clamping' to monitor the activity of single calcium channels with a microscope, and pioneered the use of two-photon microscopy for imaging the immune system.

Clarke R. Slater

After graduating from Harvard in 1962, Clarke Slater won a Marshall scholarship to study in Bernard Katz's Biophysics department at University College London where he became Ricardo Miledi's first PhD student. In addition to studies of the consequences of denervation on muscle, he worked with Ricardo at Woods Hole (1964), investigating the role of calcium in synaptic transmission at the squid giant synapse. He then spent three years in Kuffler's (S. W. Kuffler (ForMemRS 1981)) Department of Neurobiology at Harvard Medical School, five years in the Division of Cell Biology at the Medical Research Council (MRC) Laboratory of Molecular Biology, Cambridge, UK, headed by Francis Crick FRS and Sydney Brenner FRS, and one year with Terje Lømo in Oslo, working on the formation of new neuromuscular junctions (NMJs) in adult mammalian muscle. In 1975 he moved to join John Walton's Muscular Dystrophy research group in Newcastle upon Tyne, UK, eventually becoming the university's first professor of neuroscience. In Newcastle he studied the relations between the structure and function of mammalian NMJs, particularly those of humans, and how they change in disease. In addition to research papers, he wrote several substantial reviews on NMJ biology.

Stuart Cull-Candy FRS

Stuart Cull-Candy holds the Gaddum Chair of Pharmacology and is professor of neuroscience in the Department of Neuroscience, Physiology and Pharmacology at University College London. After an MSc in physiology and biophysics at UCL, he moved to the University of Glasgow to work for his PhD on invertebrate glutamate receptors. This was followed by a postdoctoral Royal Society Fellowship to work with Stephen Thesleff in Lund, Sweden. During this time he was awarded a Beit Memorial Fellowship to return to London to work with Bernard Katz FRS and Ricardo Miledi in UCL's Biophysics department. He continued for a further four years as an MRC staff member before being awarded a Wellcome Trust senior lectureship/professorship in pharmacology at UCL. His early work with Ricardo and Chuck Anderson on glutamate 'noise' and then patch-clamp studies of single-channels (with Ricardo and Ian Parker (FRS 2008)) cemented his long-standing interest in ionotropic glutamate receptors. As a pioneer of patch-clamp studies of glutamate-receptor channels he has made major contributions to our understanding of their crucial role in the brain, including the first recording and characterization of single α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor ion channels which mediate a majority of the brain's fast synaptic signalling.

Angela Vincent FRS

Angela Vincent qualified at Westminster Hospital Medical School and spent one year as a junior doctor before moving to University College London (UCL) to study basic science. With an MSc in biochemistry, she spent five years with Ricardo in Bernard Katz's Biophysics department. Moving with the myasthenia gravis research and John Newsom-Davis (FRS 1991) to the Royal Free Hospital in 1977, together they established the first neuroimmunology group in the UK. With the appointment of Newsom-Davis as Action Research Professor of Neurology in 1987, the group moved to the University of Oxford. Angela Vincent was appointed university lecturer in neuroimmunology in 1992, professor in 1998 and emeritus professor in 2016. She continued to work on autoimmune neurological diseases, discovering new antibodies to membrane proteins, examining the antibody mechanisms of action in *in vitro* and animal models, and defining the clinical features and treatment responses of the associated diseases. The five years spent at UCL with Ricardo Miledi were highly influential, forming the starting point and approach to clinical science of all her subsequent career.

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