

**OCCASIONAL PAPER****John Newsom-Davis: clinician-scientist and so much more****Angela Vincent**

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John Newsom-Davis was born in 1932 and died, aged 74, in 2007. After national service in the Royal Air Force, he read Natural Sciences at Cambridge. Following clinical studies at the Middlesex Hospital, he began research into respiratory neurophysiology with Tom Sears at the National Hospital, Queen Square, in London, and spent 1 year with Fred Plum at Cornell University in New York. After neurology specialist training at Queen Square, he became the director of the Batten Unit, continuing his interest in respiratory physiology. There he began to work on myasthenia gravis in collaboration with Ricardo Miledi at University College London and in 1978, after performing the first studies on plasma exchange in that disease, he established a myasthenia gravis research group at the Royal Free Hospital. There he investigated the role of the thymus in this disease and demonstrated an autoimmune basis for the Lambert Eaton myasthenic syndrome and 'seronegative' myasthenia. He was awarded the first Medical Research Council Clinical Research Professorship in 1979 but moved to Oxford in 1987 when he was elected Action Research Professor of Neurology. While at Oxford, he continued to run a very successful multidisciplinary group, researched further into the thymic abnormalities and cellular immunology of myasthenia, identified antibody-mediated mechanisms in acquired neuromyotonia, and began the molecular work that identified the genetic basis for many forms of congenital myasthenic syndrome. Meanwhile, he was also involved in university and college governance and contributed widely to the Medical Research Council, government committees, research charities and the Association of British Neurologists. Among many honours, he was elected Fellow of the Royal Society in 1991, appointed Commander of the British Empire in 1996 and made a Foreign Associate Member of the Institute of Medicine of the United States in 2001. Nearing and following retirement from Oxford, where he continued to see patients with myasthenia, he was the President of the Association of British Neurologists and Editor of *Brain*, and led a National Institutes of Health-funded international trial of thymectomy.

**Keywords:** antibodies; myasthenia gravis; autoimmunity

**Abbreviations:** AChR = acetylcholine receptor; ANA = American Neurological Association; CMS = congenital myasthenic syndrome; HLA = human leukocyte antigen; JND = John Newsom-Davis; MRC = Medical Research Council

**Introduction**

On a trip to Romania in August 2007, after visiting patients with myasthenia gravis in Bucharest, John Newsom-Davis (JND, as he was widely known) was involved in a fatal road accident.

This review of his life and work is based on the many tributes and obituaries that followed, JND's interview for the American Neurological Association (ANA) in 2003, personal knowledge of his work and contributions from many distinguished colleagues. His *curriculum vitae*, publications (Supplementary material B),

the ANA interview (Supplementary material C) and recollections from family, colleagues and patients (Supplementary materials D–K) are included in the online Supplementary files.

## Early years, clinical training, Queen Square and the Royal Free Hospital

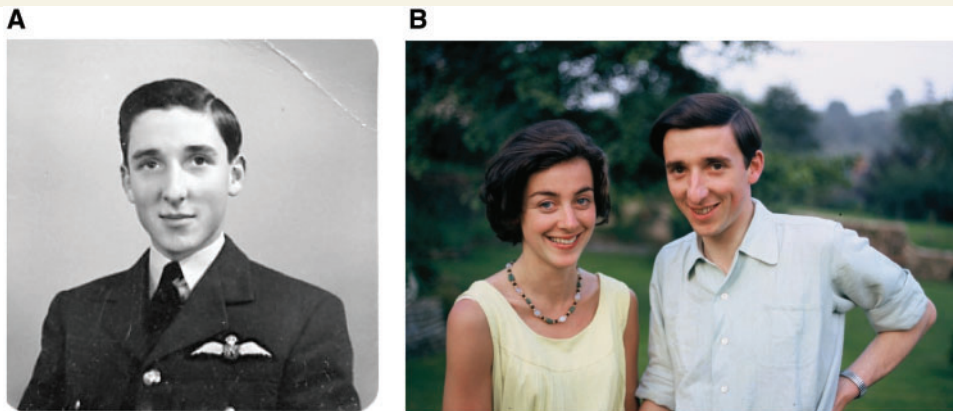
JND was born in Harpenden, Hertfordshire, UK, to John Kenneth and Dorothy Eileen Newsom Davis on 18 October 1932, older by 10 min than his twin sister Julia. The twins and their younger sister, Anthea, had a rather conventional middle-class upbringing with nannies and long walks every afternoon. He was sent to boarding school at the age of 7 years which, as a quiet and thoughtful child, he disliked intensely. At 13 years, he went on to Sherborne School, where he developed prowess in tennis and hockey, and from where he gained a place at Pembroke College Cambridge, initially to read English but changing subsequently to Natural Sciences (Supplementary material C). Though he did not see much of their father during the war, JND likely inherited his self-discipline, focus and social awareness from his father who, as managing director of the Davis Gas Cooker Company (subsequently New World Cookers), was also a prominent member of the Industrial Welfare Society and popular with his workers. JND's love of music came from both parents and his love of literature, poetry, cooking and gentleness from his mother (Supplementary material D).

JND was a member of a privileged group of 45 national service men called up in 1951 who, because of the Korean War, were offered full pilot training in the RAF during their 2 years' service. They obtained their 'Wings' at RAF Pershore in October 1953 (Fig. 1A). This was probably JND's first opportunity to show his ability to get on with people of different backgrounds—a gift that was clearly apparent, and much appreciated, throughout his career. In the early 1990s, retired British Overseas Airways Corporation Commander Dayne Markham managed to trace

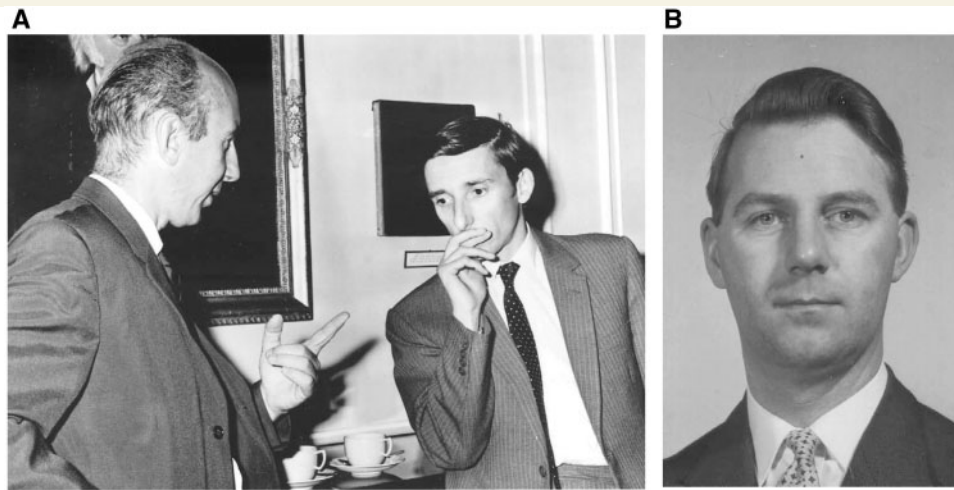
most of the 45 men and subsequently JND retained contact with his former colleagues who appreciated his unpretentious nature and interest in their lives (Supplementary material E).

At Cambridge, JND worked reasonably hard but also enjoyed university life, becoming Captain of his College hockey team, and socializing—but not particularly with the medics. During his clinical training at the Middlesex Hospital, he continued to play hockey and tennis and made frequent visits to the theatre, concerts and to the opera, with a wide and varied circle of friends (Supplementary materials C and D).

He qualified as a doctor in 1960 and, after house jobs, worked for Moran Campbell (Fig. 2A) who was very active in respiratory physiology. JND was also highly influenced by neurologists Michael Kremer (Middlesex Hospital) and Roger Gilliatt (National Hospital, Queen Square and Middlesex Hospital); together these two interests were the forerunners of his future clinical research in myasthenia. Campbell provided an introduction to neurophysiologist, Tom Sears (Fig. 2B), at the National Hospital, and JND and Sears worked very successfully together for several years. He won the Queen Square Prize in 1965 and was awarded an MD by Cambridge University in 1966, after which he was appointed to the National Hospital as a House Physician followed by University Lecturer (Fig. 3). Throughout this period he kept up his research interests, co-authoring with Moran Campbell and Emilio Agostoni *The Respiratory Muscles: Mechanics and Neural Control* in 1970—a prestigious publication for a doctor in training (Supplementary material F for the fate of one copy). In 1969, JND, newly married and accompanied by his wife, Rosemary (Fig. 1B), flew off to spend a year with Fred Plum in New York. On his return, he became a consultant neurologist at the National Hospital and took up the study of human intercostal muscle spindles. It was this preparation that led him to myasthenia gravis. 'My friend, Professor Ricardo Miledi, after distancing himself for many years from human based research, phoned to ask if I knew anyone carrying out intercostal muscle biopsies. I said "I do and it so happens he is by my side". They got together soon after and the rest is history' (Tom Sears, Emeritus Professor of Neurophysiology, King's College London).



**Figure 1** (A) 'Wings' at Pershore, aged 21 years in 1953; (B) Engagement to Rosemary in 1963.



**Figure 2** (A) With Moran Campbell at a CIBA symposium in 1969. (B) Tom Sears (date unknown).



**Figure 3** Queen Square c. 1964. JND is second from the left standing, PK Thomas is first and Roger Gilliatt third from the left in the front row.

JND and Miledi collaborated for 4 years, but in 1977, JND established an independent myasthenia gravis group in Peter (P.K.) Thomas' clinical neurosciences department at the Royal Free Hospital (Asbury, 2011). This also broke the continuity of his working relationship with Sears but there was no doubt that his time with Sears was greatly valued (ANA; Supplementary material C). As will be described in detail below, the Royal Free Hospital myasthenia gravis group grew rapidly; the first experiments were performed on the requisitioned coffee table but by 1985, generously encouraged by P.K. and his team, and largely supported by the award of two large Medical Research Council (MRC) Programme Grants (1979–84; 1984–99), the core team was formed, research assistants, students and the first tranche of visiting clinical fellows taken on, and about two-thirds of the lab space in PK's department devoted to myasthenia research.

## University of Oxford, 1987–2007

In the mid-1980s, JND was asked whether he would be interested in succeeding Brian Matthews as Professor of Clinical Neurology in Oxford. As Professor Sir David Weatherall recalls:

'One might wonder why he should have wished to move at this stage, since by this time he had been appointed MRC Clinical Research Professor of Neurology and had obtained two MRC programme grants. He had also gathered together a diverse and talented research team and the clinical environment at Queen Square, where he continued to see patients, was excellent. The question posed for Oxford, therefore,





**Figure 4** (A) At the Royal Society following election to Fellowship of the Royal Society, and (B) the Neurosciences Group in the courtyard of the Institute of Molecular Medicine in 1991.

was how he might be tempted away from this excellent environment. The temptation was the Institute of Molecular Medicine'.

In 1979, the MRC had established a Molecular Haematology Unit in Oxford directed by David Weatherall, reflecting the increasing role of molecular and cell biology for the investigation of inherited blood diseases. It was apparent that the techniques used would have wide implications across medicine, and there was a need to train young clinicians in these methods, and provide an ideal environment for clinically orientated basic scientists to work. David Weatherall attracted further MRC and other funding to create the Institute of Molecular Medicine and wanted to include neuroscience in this multidisciplinary institute. 'At first, John was quite reluctant to leave London but at a chance meeting at a research policy meeting at Leeds Castle, JND became convinced of the opportunity that a move to Oxford presented and was appointed Professor of Clinical Neurology in 1987' (Sir David Weatherall).

JND had a major impact on both medicine and the broader aspects of university life during his time as the Action Research Professor of Neurology in Oxford (1987–98). While managing an increasingly active research group, and despite innumerable calls on his time from activities outside Oxford, he maintained and expanded the excellent clinical neurology services at the Radcliffe Infirmary. He recruited neurologist Michael Donaghy and neurophysiologist Kerry Mills to Oxford as Clinical Readers, and brought Margaret Esiri and the academic work of the neuropathology department into neurology. He also appointed Peter Rothwell as clinical lecturer; subsequently Rothwell created the Stroke Prevention Unit, filling a gap left open by the departure of Charles Warlow to Edinburgh. Remarkably, in the mid-1990s, with Alan Cowey and George Radda, JND persuaded the MRC to create a functional imaging centre. They brought back Paul Matthews from London, Ontario, to direct it, and the Functional Magnetic Resonance Imaging of the Brain (fMRI) centre (now directed by Professor Irene Tracey), has subsequently become a world player in clinical and basic imaging and its applications. 'Overall, in the 11 years that he held the Chair of Clinical

Neurology, he developed a remarkably active clinical department with a research base stretching from the bedside to imaging and immunology, and numbering over 60 scientists' (Sir David Weatherall).

'John was also remarkably unselfish about giving time to help major organizational developments and activities in the medical faculty. He was a very effective member of the Board of the Faculty of Clinical Medicine from 1990–93 and chaired the Animal Care Committee at the same time. This was a period of rapid expansion of the medical faculty and John was a major influence in many directions, always giving a solid and balanced view of where the faculty should be going. He also served the University as a delegate of the University Press for 5 years, a rather terrifying body, which meets regularly each term and has the last word on which books should be published by the Press. His breadth of interests and balanced approach were of particular value to this heterogeneous group of academics. During this time John also had many commitments outside Oxford including time-demanding work for the Department of Health, the Royal Society (elected Fellow of the Royal Society in 1991, Fig. 4A), the Royal College of Physicians, and the British Association for the Advancement of Science' (Sir David Weatherall).

It was at a meeting of the British Association for the Advancement of Science in 1983 that he attracted considerable interest when he proposed that Samson had both myasthenia gravis and autoimmune alopecia (Delilah gets off the hook) and it was only following a spontaneous improvement in both diseases that Samson's hair grew and he was able to pull down the temple. The media attention that followed this retrospective diagnosis did not meet with everyone's approval.

## Medical Research Council

The 1990s were a time of change and challenges in research funding. The former CEO of the MRC Sir Dai Rees writes

'John was a wise and constructive colleague who contributed much to help MRC through difficult times. We had to learn how to think in new ways about finances, about accountability not only for the science but for political correctness'. The departures from MRC tradition threatened to dissolve the community into warring tribes—basic versus clinical, direct support versus indirect, each discipline against others, primary care and public health versus advanced diagnostics and treatment. The neuroscience community had seen great glory in the past, and would see more in the future, but the new challenge of sharing the glamour and thus the resources with brightly plumaged new cuckoos such as molecular biology and genetics, made some suspicious of the MRC's commitment. The proposed reforms of the Clinical Research Centre, then at Northwick Park, struggled under the baleful eye of the Prime Minister (Margaret Thatcher), particularly when she became persuaded that the reforms represented a threat to hospital access for her Finchley constituents. As Council Member, and particularly as Chair of the Neurosciences Board, John was in the eye of several storms. I had the impression that he found it rather fun, respecting and even admiring the enthusiasm and honest commitments of the impassioned. With his eminence and senior membership of key communities, and his cool head but personal warmth, John could put the case for the greater good with an authority and persuasiveness that outsiders to these groups could not. The MRC could hardly be in such good shape now without the dedicated and imaginative efforts of John and the allies he gathered around him' (Sir Dai Rees).

## Editor of *Brain* and President of the Association of British Neurologists

As he began to plan for his 'retirement', John agreed to take over from Ian McDonald as editor of *Brain*, a journal that John had already served as secretary and treasurer (1977–81) and as chairman of the Guarantors and Board of Management (1994–99). *Brain* had acquired a reputation for being fusty, and as the 'Queen Square house journal', but John had a knack for sniffing out work that was forward looking and contemporary; and he introduced electronic processing and on-line publication well ahead of other journals in the field (Compston; Supplementary material F).

Another change was to appoint a Deputy Editor to help, and John Rothwell (Institute of Neurology) proved to be an excellent choice.

'When John took over *Brain*, like all new editors, he wanted to bring the journal up to date and for it to lose the rather 'clubby' aura that had become, rightly or wrongly, associated with it. This didn't imply a great change in editorial policy from Ian McDonald, nor great changes in the office, but he wanted to make the journal one of the first to enter the internet age. He thought that this would broaden submissions, especially from

outside the UK, and appeal to a younger audience. He was not in favour of editorial musings or long book reviews, which at the time had tended to be dominated by a relatively small circle of neurologists, but more emphasis on the new fields of genetics and molecular biology. So we began the process of transferring from a paper-based to an electronic journal. This was in the days when most internet connections outside the university were via telephone lines, so that keeping up with submissions when on holiday or even at meetings, could be a tedious business involving dropped connections and long pauses in transmission of papers. It was also not without its teething problems, since *Brain* was one of the first large journals to be taken on by the Manuscript Central system; but for a time this was an advantage as we had a reasonable influence on how they began to develop the site.

The transition was remarkably smooth, authors and reviewers liked it and submissions began to increase dramatically. Impact factors were beginning to enter the world of scholarly evaluation and as more and more papers came in, the impact factor gradually began to rise. As editors we felt that it was necessary to try to limit the numbers of submissions for review. We stopped considering papers that did not contain clinical data, and often sent back papers if their main aim was to describe normal brain function. This meant a reduction in the number of imaging papers, and more space for genetics and molecular biology. Many thought this was an unfair policy and perhaps it was, but it had the simplicity of being highly transparent and easily understood. By the end of John's 7-year stint, we were receiving 800–900 manuscripts each year, and the acceptance rate was ~20%. It had become an unreasonable number for two editors to process, and was ripe for change when the new editor, Alastair Compston took over' (Professor John Rothwell).

JND himself recalled later (ANA; Supplementary material C):

'I wanted to try to change how the journal was perceived and what it did. John Rothwell looked after the manuscripts in neurophysiology, and we had superb support from Carol Lovelidge and Lubna Zafar. I never took a holiday from the journal for 7 years. There were no fast internet connections and in the West Indies, or wherever, my latest little grandchild vomiting down my back, being bitten by mosquitoes, perched on the edge of a bath, I was still connecting – somewhat uncertainly – with the online system via dial-up'.

Another activity was the Association of British Neurologists.

'John also took an office in Boswell Street as President of the Association of British Neurologists. As a former secretary (1981–84), medallist (1999), and the author of many papers presented over several decades, it was natural that the membership wanted John to serve as President (1999–2000). He felt that the Association had drifted away from its origins in 1932 as a forum for discussing the best of British scientific

neurology. As a first step towards bringing back the clinical scientists, he established a Clinical Research Advisory Committee to foster the clinical sciences' (Compston; Supplementary material F).

## Marriage and family life

JND's first introduction to Rosemary Schmid in 1960 was not propitious. 'I thought him much too quiet whereas he remembered someone overconfident who talked far too much' (R. Newsom-Davis, personal recollection). However, they met again at an opera party in 1963 and, with earlier prejudices forgotten, they married later that year. They settled in North London and the arrival of children followed: Amelia (1970), Imogen (1972) and Tom (1973). Rosemary worked as an educational psychologist and early family life was split between London and their weekend cottage in West Dorset. The family moved to Oxford in 1987, but following his official retirement in 1998, he and Rosemary returned to London, where the children were living and able to visit regularly. Amelia now lives in Paris and works in media marketing, Imogen is a paediatric neuropsychologist, while Tom followed his father into medicine and is an oncologist. Rosemary continues to live in West London.

'Science and medicine were never far from JND's mind. Famously he even brought a choice selection of papers with him on his honeymoon. However, when needed, he would distract himself from work through cooking (an interest he inherited from his mother), gardening and his seven grandchildren. He had a lifelong love of music, although he played no instrument himself, and regularly attended concerts and opera. Yet he was a reluctant tourist and on family holidays would usually be seen contemplating his most recent manuscript instead of admiring the relevant sights' (Dr Tom Newsom-Davis).

## Research

In the account that follows, citations of JND's work are numbered and the full references can be found in Publications (Supplementary material B). Only citations to others' publications are detailed in the text and listed in the 'References' section.

### Respiratory physiology (1965–75) and myasthenia at the National Hospital

Tom Sears, JND's MD supervisor, friend and colleague, describes their work together.

'Moran Campbell had carried out experiments testing the ability of human subjects to perceive increased resistive or elastic loading of the airways; and he had drawn on contemporary ideas of

the 'length follow-up servo control of movement' to propose imaginatively that perception depended on 'length-tension' inappropriateness to account for the results. However, the servo theory at the time conceived a muscle spindle-dependent reflex mechanism automatically subserving motor control without conscious intervention. In fact the conscious response to loading of limb muscles at that time was assigned to joint receptors with afferent pathways to the brain in the posterior columns. The neurologist Richard Godwin-Austen had recently finished a study of intercostal joint mechanoreceptors in the cat, examining these receptors as a possible source of chest wall proprioception to account for dyspnoeic sensibility. Around that time I was examining electromyographically the response of the intercostal muscles to altered mechanical loading (Sears, 1964). I therefore suggested to John that he should investigate the perception of load in patients with impaired position sense or other criteria of posterior column dysfunction. On alternate weeks John and I would impale each other with wire EMG electrodes, inserted in the external or internal intercostal muscles (sometimes with uncomfortable consequences, ANA; Supplementary material C), to examine their reflex responses to sudden changes in pressure, which could either assist or oppose the voluntary sustained lung volume, or a steadily decreasing or increasing one; similarly, a solenoid valve was used to increase or decrease the mechanical load (airway resistance). The research was based on insights gained from intracellular recordings of the central respiratory drive and the monosynaptic reflexes of intercostal motoneurons. In this way we were able to provide a comprehensive account of the segmental reflex responses of these muscles, particularly in relation to the topographical distribution of activities across the chest wall, as well as to the lung volume (% vital capacity) at which the perturbation was introduced (Supplementary materials B1 and B4). Thus our conceptual framework centred on the muscle spindle and John, who had access to human intercostal muscle biopsies, subsequently used the same experimental rig to examine the muscle spindles in these muscles, and provided the first account of their activation in his *in vitro* preparation (Tom Sears; Supplementary material B8).

From this experience John gained invaluable experience in electrophysiological measurements and subsequently became very interested in hiccup (Supplementary material B3), and described a new method to measure conduction velocity of human phrenic nerve fibres, which hitherto had depended on oesophageal electrodes (Supplementary material B2). Surface electrodes placed over the insertion of the diaphragm in the lower ribs provided clean EMG signals in response to stimulation of the phrenic nerve in the neck, thus providing objective data where the assessment of paradoxical movement was equivocal.

In New York with Fred Plum from 1969–70, he carried out experiments on the anaesthetized cat to determine the trajectory of descending bulbospinal pathways responsible – firstly, for the 'central respiratory drive' to respiratory motoneurons in the spinal cord, and secondly, for those responsible for the cough reflex (Supplementary material B5). Both questions had their origin in work done at Queen Square, namely that of Peter



Nathan on the pathways in man, as deduced from the effects of cervical tractotomy for the relief of intractable pain; and our studies on the effects of selective lesioning and stimulation experiments in the medulla studied. The experiments were meticulous and with excellent histological control to allow clear conclusions concerning the innervations of the diaphragm and abdominal muscles. Those responsible for the cough lay in the ventral quadrant of the cervical spinal cord, whereas those carrying the respiratory drive were in a ventrolateral location, just as Nathan had found for man (Supplementary material B5). These feline studies epitomized John's interest in seeking experimentally to discover mechanistic explanations to illuminate his clinical work.

John's links with the emerging Department of Neurophysiology continued while he was Physician-in-Charge of the Batten Unit. He collaborated with the lecturer in Bioengineering in that department, David Stagg, who developed a comprehensive computer-based analysis of airflow and tidal volume in eupnoea to reveal a complex breath-by-breath variation in such individual parameters as inspiratory and expiratory durations, cycle time, and their correlation with tidal volume (Supplementary material B9). Nevertheless, this seemingly random variability was such that mean inspiratory flow rate was held constant, the work providing a fresh insight concerning the homeostatic regulation of breathing. Mike Goldman, an expert on the use of 'magnetometers' for measuring body wall displacements, then at the Harvard School of Public Health, was encouraged to come to Queen Square. This led to a further collaboration in which John, David Stagg and Mike Goldman were able to show that magnetometers placed over the ribcage and abdomen to measure changes in their anterior/posterior diameters provided signals that, coupled to a calibration procedure, allowed the assessment of respiratory function without the need for face masks, a valuable method for sick patients' (Tom Sears; Supplementary materials B10 and B14).

JND did not entirely neglect this promising early work. Over the next 10 years or so, many clinical studies on patients with compromised ventilation were undertaken, many with anaesthetist Laurie Loh, who subsequently moved with JND to Oxford (Supplementary materials B10, B11, B13, B14, B15, B24 and B25).

## Myasthenia

It was the intercostal muscle preparation that, in 1973, while Physician-in-Charge of the Batten (Intensive Care) Unit, changed the direction of JND's career. As mentioned above, Tom Sears had been contacted by Ricardo Miledi, an electrophysiologist of great distinction who had spent most of the preceding 20 years with the 1969 Nobel Laureate (later Sir) Bernard Katz, in the Biophysics Department at University College London. Together they described many aspects of the physiology of neuromuscular transmission in species from squid to man, including seminal work on the role of calcium in acetylcholine release, and had just published the first observations on the

electrical 'noise' that resulted from application of acetylcholine, and which provided data that could be used to calculate the behaviour of single acetylcholine receptor (AChR) ion channels (Katz and Miledi, 1970).

Miledi was medically trained and had followed the work of Elmqvist and colleagues (1964) showing reduced amplitude of the miniature endplate potentials in muscles from patients with myasthenia gravis, a disease that had been thought to affect neuromuscular transmission since Jolly's first electromyographic description (Jolly, 1895). Those observations did not fully resolve the question as to whether the defect was presynaptic—due to reduced release of acetylcholine—or postsynaptic, potentially due to defects in the AChR itself. Miledi had recently used the snake toxin  $\alpha$ -bungarotoxin, originally discovered by Chang and Lee (1963) in Taiwan, to quantify and purify AChRs from 'Torpedo' electric organs and from rat muscle (Miledi *et al.*, 1971). It seemed likely that bungarotoxin could be used to analyse AChRs at myasthenia gravis endplates—but he needed to find someone who could provide human muscle biopsies. JND, as part of his work in the Batten Unit, frequently had patients with myasthenia gravis under his care and, with an introduction from Tom Sears, a highly successful collaboration was quickly established. Intercostal muscle samples were obtained at thymectomy with minimal risk to the patients. Control muscle samples were available from the London Chest Hospital, mainly from patients undergoing thoracotomies, through the help of chest surgeon Mr Marvin Sturridge who also did the thymectomies at Queen Square.

Within a few weeks, the first muscle biopsies began to arrive at UCL; endplate recordings were made by visiting postdoc Yushi Ito who confirmed that  $\alpha$ -bungarotoxin inhibited human muscle neuromuscular transmission and that the miniature endplate potentials were indeed substantially reduced in amplitude at myasthenia gravis endplates. The remaining muscle was immersed in radioactive  $^{125}\text{I}$ - $\alpha$ -bungarotoxin and the muscle fibre bundles dissected into small sections containing the endplates. Counting these for radioactivity showed that the number of  $^{125}\text{I}$ - $\alpha$ -bungarotoxin-binding sites, and thus the AChRs, were substantially reduced, which was confirmed by autoradiography. Unfortunately, Fambrough *et al.* published their very similar results in *Science* in 1973. Having been thoroughly scooped, JND and Ricardo concentrated on making their study more comprehensive, and it was published in *Brain* in 1978 (Supplementary material B18).

Meanwhile, another visiting postdoc Peter Molenaar, had shown that acetylcholine was increased in myasthenia gravis muscle, and in rats treated with bungarotoxin or immunized against AChRs (Supplementary material B26). These observations implied that, in the presence of reduced AChR function, there might be compensatory increases in acetylcholine synthesis and release—findings that were later confirmed when Molenaar, visiting the Royal Free Hospital, showed an increase in choline acetyltransferase, the enzyme responsible for acetylcholine synthesis (Supplementary material B34), and subsequently investigated in more detail in both myasthenia gravis and AChR-immunized rats (Plomp *et al.*, 1994).

## Plasma exchange and treatments for myasthenia gravis

During the mid-1970s JND continued to work on intercostal muscle spindles, including describing the endplate morphology in myotonic dystrophy with his postdoc David Strannock (Supplementary materials B21 and B22). The move to concentrate on myasthenia gravis followed the exciting developments in the USA where antibodies to AChRs were first identified both in animals immunized against purified receptors (Patrick and Lindstrom, 1973) and in patients with myasthenia (Lindstrom *et al.*, 1976), and the demonstration that injection of IgG purified from patients with myasthenia gravis reduced miniature endplate potentials and AChR numbers, and caused evident weakness, in mice (Toyka *et al.*, 1975). Extrapolating from the use of plasma exchange in patients with Goodpasture's syndrome, Tony Pinching and Keith Peters tried the treatment on two patients with acquired myasthenia gravis and one with congenital myasthenia (Pinching and Peters, 1976); their observations were extended by Pinching, (Newsom-)Davis and Peters in 1977 (Supplementary material B17). The rapid (within 1–2 days) and impressive temporary improvement obtained by simply removing a large proportion of circulating plasma proteins was probably what convinced JND to move from respiratory neurophysiology and concentrate on myasthenia gravis. He obtained a project grant from the MRC, recruited A.V. who had been doing some of the myasthenia gravis work in Miledi's laboratory, and set up the research group at the Royal Free Hospital with much support and enthusiasm from P. K. Thomas (Vincent; Supplementary material G).

During this time JND showed his adaptability by taking Ivan Roitt's "post-experience immunology" evening course so that he was soon fully au fait with this field.

## Myasthenia at the Royal Free Hospital

JND's approach to the research that began at the Royal Free Hospital and subsequently transferred to Oxford, was that few projects were completed and then dropped—almost every disease area was pursued and extended, or taken up again when more suitable techniques became available. His achievements (Table 1), which were based on building up a stable multidisciplinary team, involved all of the diseases of the neuromuscular junction and the history of his work on each of these conditions stretches over

some time (Fig. 5). In the passages that follow, most of which represent many overlapping years of endeavour, the scientists and clinical fellows who performed the work will be named. This is not to diminish JND's contributions—in fact, he was always deeply involved with everything going on—but to show that he chose able scientists and clinicians, encouraged visitors from around the world, and inspired enthusiasm and commitment, while allowing the growing team a good deal of independence. As a result, the team survived after his retirement and several aspects of the work have continued, as will be briefly recounted below.

The questions that first needed to be answered were obvious. How much did the antibodies fall after plasma exchange, and did their levels correlate with clinical indices of disease? The results in seven patients, including one with congenital myasthenia who had no detectable AChR antibodies and did not improve clinically, otherwise showed a remarkable inverse correlation—not only as the AChR antibodies fell following the exchanges, but also as they subsequently rose (Supplementary material B19). At this time the main additional immunosuppressant was azathioprine, following the pioneering work by Mertens *et al.* (1969). AChR antibody levels fell only slowly after azathioprine [although significantly faster in patients with thymoma than those without (Supplementary material B28)], perhaps explaining why there appeared to be no extra benefit or synergy of plasma exchange combined with immunosuppression (Supplementary material B28). It was many years before JND and Jackie Palace performed the seminal trial of prednisolone alone versus prednisolone plus azathioprine (Supplementary material B212) demonstrating that azathioprine reduced the maintenance dose of prednisolone required to achieve clinical remission, but that this effect took 10 months to be evident. Not only has the combination of azathioprine and prednisolone become the main first line immunosuppression in myasthenia gravis: the trial approach has been adopted for other studies in myasthenia gravis.

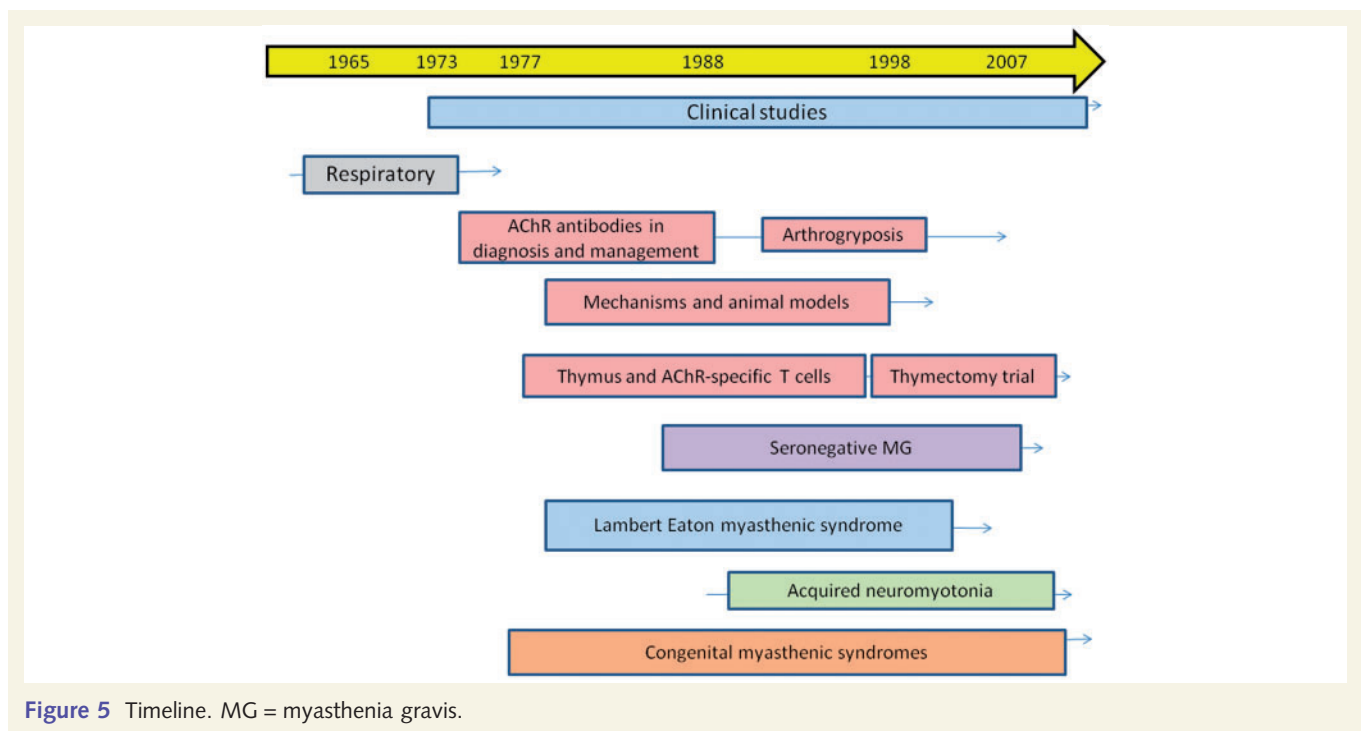
## The thymus and antibody synthesis

One of the first projects JND became enthusiastic about at the Royal Free Hospital was the study of the thymus for which he joined forces with Glenis Scadding, a clinical fellow working with Howard Thomas, at that time a clinical lecturer in Dame Sheila Sherlock's renowned Hepatology Department. Scadding was culturing thymic tissue explants in order to test for AChR antibody production. Indeed, the antibodies were present in the culture

**Table 1** Defining the clinical spectrum, molecular mechanisms and treatment responses in neuromuscular junction disorders

Establishing the importance of plasma exchange for treatment and for identification of antibody-mediated mechanisms.
Exploring autoantibody-mediated pathogenesis by experimental <i>in vitro</i> and <i>in vivo</i> studies.
Identifying target molecules and devising more specific diagnostic antibody assays.
Describing immunogenetics, and the immunopathology of the thymus and antigen-specific T-cell responses.
Establishing the genetic basis and molecular targets in inherited forms of myasthenia.
Developing systematic flow charts for diagnosis and treatment.





supernatants, though their detection required highly sensitive assay conditions. A preliminary report appeared while Scadding was on maternity leave (Supplementary material B23), but she subsequently wrote a full report after longer gestation (Supplementary material B38). The production of antibodies by the thymus—only seen in those patients with a ‘hyperplastic’ thymus—was an exciting, although at first controversial, finding. When presented with the early data, leading immunologist Av Mitchison walked out of the room muttering something about ‘the thymus doesn’t contain B cells and doesn’t produce antibodies’! Nevertheless, it prompted recruitment of a real immunologist, Nick Willcox, allowing many future studies on the thymus, the T, B and plasma cells (see below), but also on the effects of thymectomy on antibody levels. Despite several publications from other groups claiming no convincing effect, careful analysis showed a clear influence of thymectomy alone on AChR antibody levels, which declined on average by ~50% over a year in patients with hyperplastic thymi (Supplementary material B53), but not in thymoma patients, as predicted from their poorer clinical response.

## Human leukocyte antigen and disease diversity

One of his most cited myasthenia gravis papers resulted from JND’s collaboration with a trainee at Queen Square, Alastair Compston, and his PhD supervisor Richard Batchelor. Batchelor had been involved with the early blood grouping and tissue typing studies at East Grinstead, and recognized their value in demonstrating genetic associations with autoimmune diseases.

Compston was working principally on multiple sclerosis but enthusiastically undertook to analyse 68 blood samples from patients with myasthenia looking, as one had to in those days, for evidence of human leukocyte antigen (HLA) type by serological cross-matching of fractionated T and B lymphocytes. The results confirmed the high incidence of the HLA B8-DR3 haplotype in myasthenia; but it was restricted to younger patients, whereas HLA B7-DR2 was more frequent if onset was after the age of 40 years (Supplementary material B30). Thymoma cases, considered separately for the first time, did not show a specific HLA association. This study has not only stood the test of time but clearly indicates the importance of age and pathology stratification of patients when analysing their genetic associations—even when the final disease mechanisms, AChR antibodies in this case, are the same.

JND’s work became an attraction for visiting fellows and several contributed over the next few years. Ho-Chang Chiu came from Taiwan with sera from his patients with myasthenia gravis who were younger than the Caucasians, a high proportion presenting at <10 years of age. These were both males and females and mostly had ocular or mild myasthenia, with relatively low AChR antibodies (Supplementary material B83). There were also immunoglobulin allotype differences in Japanese patients, who showed an association, that Taiwanese or Caucasians did not (Supplementary material B95). Clinical fellow, Stephanie Robb, found another difference from asymptomatic Japanese subjects who reportedly showed raised levels of antibodies to AChR, which she did not find in a small study of elderly individuals from the UK (Supplementary material B71). Both of these studies have been followed up more recently with analyses of myasthenia

gravis in mainland China (Zhang *et al.*, 2006; Yang *et al.*, 2010), and of AChR antibodies in older people with and without diagnosed myasthenia gravis (Vincent *et al.*, 2004).

## Antibody characterization

The first full study of AChR antibodies in samples sent for routine serological assays ( $n = 2967$ ) was not published until 1985, when it compared the levels of antibodies with those found in 153 validated cases from JND's clinic (Supplementary material B73). Meanwhile, detailed characterization of the antibodies and the antigenicity of the AChRs were described in patients with generalized, ocular or penicillamine-induced myasthenia, with recent onset of disease, or with very low AChR antibodies. This work highlighted the great heterogeneity of the antibodies in affinity, subclass, light chain isotype, cross-species reactivity, competition with monoclonal antibodies (see below) and reactivity with different epitopes on AChR from normal, denervated and ocular muscles. It also indirectly demonstrated an antigenic difference between normal and denervated (foetal) AChRs and even between the two bungarotoxin-/acetylcholine-binding sites (Supplementary materials B45, B46, B54 and B74).

By 1979, JND had recruited a new postdoc, Bethan Lang (from Eric Barnard's laboratory at Imperial College London), to purify the patients' antibodies by affinity chromatography (Supplementary material B41). Beth proceeded to study anti-idiotypic antibodies—which bind specifically to the antigen-combining site or other unique epitopes on the AChR antibodies themselves—by immunizing rabbits with the purified antibodies. However, the resulting 'anti-idiotypic' antibodies did not cross-react with AChR antibodies from other patients except in one case (Supplementary material B66), indicating that most patients' antibodies were idiotypically distinct.

The next recruit was Paul Whiting, JND's first MRC PhD student; this was very fortunate as Paul was an exceptionally modest but effective young man who later worked with Jon Lindstrom at the Salk Institute, returned to Pharma in the UK and is now Executive Director and Site Lead of Pfizer's Regenerative Medicine Research Unit. Whiting purified human AChRs from amputated limbs—a gruesome business that often meant travelling around London on the underground or buses carrying large smelly bundles wrapped in black plastic bags. He produced monoclonal antibodies against the human AChR that he then used to characterize the AChRs and their antibody-binding sites by competition assays. Not unexpectedly, Whiting's monoclonal antibodies proved to be highly specific for different regions on human AChR isoforms, rather more discriminating than most raised against 'Torpedo' electric organ by Socrates Tzartos working with Jon Lindstrom and colleagues (1981). In particular, four monoclonal antibodies bound only to AChRs from denervated (extrajunctional/foetal type) muscle and not from the adult neuromuscular junctions (Supplementary material B82). This confirmed the molecular distinction, demonstrated by the cloning of the genes in 1985 (Shibahara *et al.*, 1985). These monoclonal antibodies also allowed A.V. to convince herself of the absence of anti-idiotypic antibodies in myasthenia gravis, challenging the

prevailing hype (Vincent, 1981). Later a PhD student, Andy Roberts (now at Cambridge Antibody Technology), induced anti-idiotypic antibodies against some of the monoclonal antibodies, but they did not cross-react with human AChR antibodies (Supplementary material B147).

Whiting, and subsequently visiting clinical fellow, Fedor Heidenreich, used these monoclonal antibodies in further competition experiments to characterize the antibodies in thymoma, early- and late-onset groups and in serial samples during treatment (Supplementary materials B75, B81 and B98). Heidenreich went on to show close similarities in specificities between the patient's antibodies in thymic culture supernatants and serum pre- and post-thymectomy (Supplementary material B100), indicating that the thymus was indeed a likely source of the full repertoire of antibodies. Some years later, clinical training fellow Jeremy Farrar (now Director of the Oxford University Clinical Research Unit in Ho Chi Minh City, Vietnam), supervised by Nick Willcox, was able to recombine the heavy and light chain genes from messenger RNA expressed in the thymus of a patient with myasthenia gravis and demonstrate the diverse germ-line gene origins of the antibodies (Supplementary material B191).

## Disease mechanisms

Much had been learnt about disease mechanisms in the 1970s from the laboratories of Andrew Engel, Jon Lindstrom, Dan Drachman, Zach Hall and many others. These had shown that the antibodies could inhibit acetylcholine or  $\alpha$ -bungarotoxin binding to rodent receptors (Weinberg and Hall, 1979), and reduce the surface expression of AChRs on cultured cell lines by a process known as antigenic modulation—cross-linking and internalization of the receptors followed by their degradation (Stanley and Drachman, 1978). And perhaps most importantly, by complement-dependent damage to the receptor-containing postsynaptic membrane following antibody binding (reviewed by Engel *et al.*, 1984). These three mechanisms are still thought to be the most important in AChR antibody-positive myasthenia gravis. Steve Wilson, JND's first clinical fellow, asked whether antigenic modulation really operated at the neuromuscular junction where the natural turnover of AChRs is very slow (half-life 10 days). By injecting radioactive  $\alpha$ -bungarotoxin into pleural cavities of mice that had been injected systemically with myasthenia gravis antibodies, he was able to label the diaphragm AChRs and show that they did indeed turnover faster (see also Stanley and Drachman 1978), but, unexpectedly, their synthesis rate also increased. These were highly relevant findings at the time, but having been turned down by *Nature* and with Wilson returning to neurology as a Senior Registrar, they were published a little less prestigiously (Supplementary materials B56 and B57).

There are still, however, several unexplained effects of myasthenia gravis serum on neuromuscular junction functions. Andy Lerrick, a visiting medical student from the USA, joined Dennis Wray, a lecturer at the Royal Free Hospital (now recently retired as Professor of Biophysics at Leeds University), an experienced electrophysiologist who began a very fruitful collaboration with JND (Ricardo had left London for the University of California at

Irvine, where he is still experimenting and publishing). A rapid inhibitory effect of myasthenic serum on miniature endplate potential amplitudes was found; this was heat-sensitive, not apparently in the IgG fraction, and its nature has never been adequately explained (Supplementary material B49).

## Cloning the acetylcholine receptor genes and different human acetylcholine receptor isoforms

By 1986, genetic forms of myasthenia were beginning to be studied and it was clear to JND that this would be a fruitful area for the future. He recruited David Beeson who, like Bethan Lang, had done his PhD with Eric Barnard at Imperial College, expressly to clone and sequence AChR genes, supported by a grant from the Muscular Dystrophy Group (now Campaign) to JND and Barnard. The first Torpedo and Electric eel AChR subunits had been cloned in Barnard's laboratory, in Japan and in the USA, and Beeson had already managed to describe chicken AChR gene sequences and expression during his PhD, but JND wanted the human sequences. It is hard to appreciate how difficult this was in those days, with no kits, and techniques that had to be composed more or less from scratch. But not only did JND want to be able to study patients with congenital myasthenic syndromes but AChR polypeptides were needed for Willcox's T-cell studies (see below).

The first human subunit to emerge from Beeson's work was the  $\beta$  (Supplementary material B109), and then he and postdoc Alison Morris discovered a novel exon of the human  $\alpha$  subunit, which was generated by alternative splicing and did not appear to be present in other species, apart from close primates. This encoded an extra 25 amino acids and the two isoforms could be distinguished by means of antibodies to the additional 25 amino acids, raised in rabbits by research assistant Leslie Jacobson (Supplementary material B132). The two isoforms appeared to be equally expressed in different human muscles (Supplementary material B151) and also in the thymus. However, regrettably the expression of the isoform with the additional 25 amino acids did not result in a functional AChR when the complementary DNAs were injected into *Xenopus* oocytes by postdoc Claire Newland (Supplementary material B178), and to date, no role for this quirk of human genetics has been found either in neuromuscular junction function or in myasthenia.

Meanwhile, Beeson had also produced the first primary complementary DNA sequences for the  $\gamma$  and  $\epsilon$  subunits of the human AChR, which by that time were realised to define the foetal and adult AChRs, respectively (Supplementary material B149), and he had also identified the chromosomal localization for the human  $\alpha$ ,  $\beta$ ,  $\delta$  and  $\epsilon$  subunits (Supplementary material B120). This, and the growing expertise in RNA as well as DNA technologies, enabled clinical training fellow Cal MacLennan to study the expression of the foetal and adult-defining subunits in human extraocular muscles (Supplementary material B194), confirming the earlier observations suggesting that these muscles did not contain large amounts of foetal AChRs despite their multiply-innervated muscle fibres (Supplementary material B46), and questioning the hypothesis put forward by Henry Kaminski and Robert Ruff (1997)

that ocular myasthenia gravis was due to antibodies specifically directed at the foetal AChRs. Indeed, the extraocular muscles turned out to express a high concentration of RNA for the adult-specific  $\epsilon$  subunit, and this suggested that adult AChR might be an important target for antibodies in patients with purely ocular myasthenia gravis, as was shown by comparing their serum reactivity with AChRs extracted from muscle cell lines that had been engineered to produce large amounts of either foetal or adult isoform (Supplementary material B194).

Use of the two isoforms, and their expression in *Xenopus* oocytes, enabled the group to investigate a rare but devastating condition. A few mothers with myasthenia gravis had been reported to have babies born with arthrogryposis multiplex congenita—multiple joint contractures, often with other deformities and resulting from lack of foetal movement. JND, David Hilton-Jones, a consultant neurologist in Oxford, with Jane Hurst and other clinical geneticists, had diagnosed myasthenia gravis in a mother with four consecutive affected babies, three of whom had died perinatally (Supplementary material B170); moreover, another female without any history of myasthenia gravis had five affected pregnancies (Supplementary material B181). This suggested that AChR antibodies in these unfortunate females were crossing the placenta and paralysing the foetus during development; their ability to inhibit the function of foetal AChRs, while leaving the adult form unaffected (although still a target for some less inhibitory antibodies), was convincingly demonstrated by Claire Newland, David Beeson and visiting medical student Sietske Riemersma (Supplementary material B189). The role of the antibodies in this condition was formally confirmed by Leslie Jacobson and visiting paediatric fellow Agata Polizzi, by injecting pregnant mice with the antibodies and showing arthrogryposis and perinatal death in the offspring (Jacobson *et al.*, 1998). Some time later, Ian Matthews, a postdoc supported by the Association Francaise contre les Myopathies, made a recombinant library from the thymus gland of the original mother; the cloned and recombined antibodies were highly mutated and almost entirely specific for foetal AChRs (Matthews *et al.*, 2002).

## Expanding the immunology—animal models, thymus, germinal centres and myoid cells

### The thymus and antibody formation

The finding of antibody synthesis by the thymus was exciting. Fortunately, JND was able to convince the scholarly and talented (albeit somewhat eccentric) immunologist Nick Willcox, who like JND had qualified at the Middlesex Hospital, to abandon a tenured lectureship in anatomy in Newcastle in 1979 and join the team at the Royal Free Hospital, supported by JND's newly awarded MRC programme grant. Willcox set about investigating the cells that were involved in the thymus and also in the blood, where a small amount of antibody synthesis could also be stimulated with mitogens, as demonstrated in preliminary experiments by neurologist Charles Clarke. Postdocs Gillian Harcourt and Andy Jermy, together with research assistant Chris Fisher, began to explore antigen-specific T cells and animal models.



Glenis Scadding made a surprising finding (Supplementary material B80). Lindstrom, Lennon, Patrick and their co-workers had immunised mice actively with electric fish AChR emulsified in the standard strong adjuvant. But Scadding found that she could induce antibody levels against mouse AChR by injecting mouse AChR-expressing membranes intrathymically or intraperitoneally without adjuvant. These antibodies were at least partially pathogenic, since miniature endplate potentials were modestly reduced in amplitude in the mice studied by Dennis Wray and his student Chris Prior (Supplementary material B80). These surprising findings were explored in different strains of mice by Andy Jermy using adoptive transfer approaches to prove that the antibodies were T-cell-dependent (Supplementary material B111); BALB/c mice injected with mouse AChR often became overtly weak with antibodies very similar to those found in human myasthenia gravis (Jermy *et al.*, 1993).

Although the mice in these and other animal models showed no abnormalities in the thymus, its involvement in the human disease became a major focus of JND and Willcox's work over the next few years. This began at the Royal Free Hospital with crucial help from Professor George Janossy and his Immunology Department where immunohistological studies began to dissect the role of the medullary germinal centres, so similar to those found in peripheral lymphoid tissue. The germinal centres correlated with AChR titre and thymic antibody synthesis, suggesting the presence of both CD4 lymphocytes and antigen-presenting cells in the medulla; and the accessibility to peripheral-type T cells appeared to create opportunities for their autoimmunization (Supplementary materials B44 and B63), though it was not clear what was initiating the aberrant development of germinal centres. One early possibility was viral infection of the thymus, but investigations by PhD student, Linda Klavinskis, argued strongly against that (Supplementary materials B64 and B65). It had been suggested, however, that AChR was present in the rare medullary thymic myoid cells, and this was confirmed when visiting clinical fellow Myriam Schlupe used the AChR monoclonal antibodies to stain the thymus (Supplementary material B90). Despite their rarity, the myoid cells were often close to the germinal centres, implicating them in their formation (Supplementary material B202), and thus in autoantibody diversification. Indeed, postdoc Ian Matthews with visiting clinical fellow Hiro Shiono, together with Glasgow immunologists Gary Sims and David Stott, subsequently showed that the IgGs cloned from the thymic B cells were highly mutated (Supplementary material B278; Shiono *et al.*, 2003a). An important observation, not widely appreciated, was that pre-treatment of the patients with steroids altered the thymus and thymoma architecture and cell populations, particularly the cortical lymphocytes (Supplementary material B118).

In the 1990s, several groups detected expression of isolated AChR subunits in thymic epithelial cells from non-thymoma patients (Wakkach *et al.*, 1996; Supplementary material B279). As a result of a collaboration between David Beeson, Henri-Jean Garchon (Paris) and Bruno Kyewski (Heidelberg), a predisposing polymorphism of the AChR  $\alpha$  subunit gene was found to influence AChR expression in the epithelial cells, particularly under the influence of a protein called the Autoimmune Regulator (AIRE; see below; Giraud *et al.*, 2006).

## Thymoma

Collaborating with Mary Ritter (Royal Postgraduate Medical School), JND and Willcox also scrutinized myasthenia gravis-associated thymomas, noting combined expression of cortical and medullary markers on the neoplastic epithelial cells and excess generation of maturing T cells (Supplementary material B92), many of which clinical training fellow Camilla Buckley showed are exported to the periphery (Supplementary material B233). They proposed the common ancestry of both normal and neoplastic thymic epithelial subsets that is now generally accepted. How the thymoma induces AChR antibodies was hotly debated but, despite claims from others, neither expression of whole AChR nor spontaneous synthesis of AChR antibodies was detected in thymomas (Shiono, 2003a, b), although clinical training fellow Cal MacLennan and David Beeson found high AChR  $\varepsilon$ -subunit expression (Supplementary material B279), and research assistant Nita Nagvekar cloned AChR-specific T cells from two myasthenia gravis thymomas (Supplementary material B209).

In later studies with Tony Meager at the National Institute of Biological Standards and Control (Supplementary materials B225, B249 and B255), however, it was found that antibodies were present in thymoma patients that could neutralize interferon- $\alpha$ 's and interleukin 12, and Camilla Buckley showed that these antibodies often increased substantially in the circulation when thymomas recurred (Supplementary material B234). The presence of these neutralizing antibodies may well explain the susceptibility to infections frequently found in association with recurrent thymomas. By a happy coincidence, much later Meager and Willcox identified similar antibodies in the monogenic autoimmune polyendocrine syndrome-I (APS-I, alias APECED) (Meager *et al.*, 2006; Kisand *et al.*, 2010), which is due to mutations in *AIRE*, that normally ensures that medullary thymic epithelial cells express low levels of peripheral autoantigens like insulin and AChR- $\alpha$ , presumably to tolerate T cells.

## Acetylcholine receptor-specific T cells

The production of AChR antibodies in the myasthenia gravis thymus, and their correlation with thymic histology (Supplementary material B38), suggested a primary role for locally expressed AChR (Supplementary material B36). Nevertheless, some AChR antibody production continued after thymectomy, so there must also be peripheral synthesis. This was the major focus of JND's early work at the Royal Free Hospital where evidence of control by specific T cells exported from the thymus was pursued by investigating the cell types involved in both thymus and blood (Supplementary materials B55 and B62), and in serial studies of patients with myasthenia gravis (Supplementary material B69). For this it was important to have purified AChRs initially obtained from the electric organs of '*Torpedo*'. Visiting clinical fellow Norbert Sommer found that AChR-reactive T cells were present in both patients and controls (Supplementary material B137), probably indicating incomplete deletion of autoreactive T cells in the thymus. Moreover, both myasthenic thymi and thymomas appeared to be selectively enriched in AChR-reactive T cells (Supplementary material B129).

With the cloning of the human AChR subunit genes by David Beeson, and the growing use of synthetic peptides (firstly from

collaborator Dr Jonathan Rothbard), Gillian Harcourt and visiting neurologist Ben Ong, first stimulated specific T cells from patient thymus or blood with recombinant human AChR polypeptides, carefully purified by Nadia Pantic, cloning the responding T cells, and then mapping the epitopes with unusually long, and then shorter overlapping peptides, made by Leslie Jacobson. One AChR-specific T-cell clone from an HLA-DR3/4 heterozygous early-onset myasthenia gravis patient's thymus demonstrated a role for a recurring <sup>86</sup>Gly↔Val dimorphism in the presenting HLA Class II molecules (a collaboration with John Bell and Paul Wordsworth in the Human Immunology Group at the Weatherall Institute of Molecular Medicine; Supplementary material B134). This was explored further by visiting neurologist Simon Hawke, who found that the <sup>86</sup>Gly allowed binding of anchor residues with bulkier side-chains like the <sup>149</sup>Trp of the AChR 147–160 epitope; surprisingly this was recognized only when presented by DR4, especially the DR4 subtypes that predispose to rheumatoid arthritis (Hawke *et al.*, 1999), and not by the common myasthenia gravis-associated DR3. Possible relevance to myasthenia gravis, however, was shown by visiting postdoc Fulvio Baggi who demonstrated that the same clone responded to its epitope when processed and presented from the endogenous AChR by an HLA-DR4-transfected TE671 AChR-expressing cell line (Supplementary material B148), while visiting neurologist Nils Erik Gilhus showed that thymoma epithelial cells could also present this and other peptide epitopes to specific T cells (Supplementary material B173).

Despite these early successes, the team soon encountered a major problem. The recombinant AChR subunits were made from *Escherichia coli*; it was much easier to clone T cells against the *E. coli* contaminants than against 'real' epitopes processed naturally from whole AChR (Supplementary material B176). Responses to shorter peptides were probably not the answer as they were not naturally processed. The visiting overseas clinical fellows (including Mike Nicolle, Hidenori Matsuo and Anna-Paola Batocchi) carefully compared the specificities of the T cell lines with clones they had raised in parallel against synthetic AChR peptides, rather than recombinant subunits. The responses to native AChR, captured by monoclonal antibodies onto immunomagnetic beads, demonstrated which clones were truly AChR specific (Supplementary material B144). In contrast, T cells selected initially against synthetic peptides showed no detectable responses to whole AChR (Supplementary material B176), probably because the T cells had been selected against peptides loaded unnaturally into Class II molecules on the surface of the antigen-presenting cells rather than via the endosomal pathway.

More encouragingly, clinical training fellow Marguerite Hill starting with recombinant  $\epsilon$  subunit isolated clones responding to an extracellular human AChR  $\epsilon$ 201–219 epitope from three of four patients with early-onset myasthenia gravis and one with D-penicillamine-induced myasthenia gravis (Supplementary materials B217 and B218). Overall, the eight native AChR-specific clones that were identified all recognized extracellular AChR epitopes and were presented to the different T-cell receptors often by 'minor' Class II molecules (HLA-DQ, -DP and -DR52a) rather than the HLA-DR molecules that associate strongly with myasthenia gravis. The results of these long years of toil strongly suggested

that specific T cells in patients with chronic autoimmune diseases are rare, and questions the ease with which others have identified 'specific' responses to short peptides or intracellular proteins in other diseases.

The identification of a potentially dominant pathogenic epitope raised the possibility of a 'magic bullet' approach for selective therapy in myasthenia gravis. Visiting neurologist Mike Nicolle (Supplementary material B166) found that cloned T cells could be substantially inhibited by pre-treatment with specific soluble peptide: HLA-DR4 complexes without co-stimulation (prepared by collaborators at Anergen, a US Biotech company). Moreover, they induced apoptosis in the T cells, and thus seemed promising as long-term therapeutics. This was later taken up by postdocs Alison Bond, Louise Corlett and John Curnow to define more precisely the underlying mechanisms and to explore alternative strategies (Supplementary material B203), e.g. using AChR $\epsilon$  peptide: Class II tetramers. Unfortunately, the MRC Programme grant came to an end with JND's retirement, and the project never came to fruition.

One other aspect that was not taken forward was the study of HLA Class I. The HLA associations in early-onset myasthenia gravis span the HLA-DQ2-DR3-B8-A1 haplotype, but are consistently stronger with the Class I molecule-B8 than with -DR3 (Supplementary material B220). John Curnow focused on CD8-mediated immune responses towards human myoblasts, hoping to explain auto-sensitization *in vivo* and the role of Class I in disease susceptibility (Curnow *et al.*, 1998, 2001).

## Congenital myasthenic syndromes

One of the striking, but not altogether unexpected, observations that JND made in the 1970s was that patients with myasthenic symptoms from early childhood did not respond to plasma exchange [and did not have AChR antibodies (Supplementary material B29)]. Meanwhile Andrew Engel in the Mayo Clinic had begun to study patients with inherited, presumed genetic, conditions including the Slow Channel Syndrome, in which the duration of miniature endplate potential and endplate potential amplitudes are grossly abnormal, resulting from prolonged openings of the individual AChR ion channels (Engel *et al.*, 1982). This is often a slowly progressive disease that may present in adulthood, as described in two cases by JND with Hans Oosterhuis in Groningen (Supplementary material B89). In general, patients with congenital myasthenic syndromes (CMSs) can develop arthrogryposis *in utero*, be floppy or intermittently weak from birth, or develop weakness later on. Although often not progressive, some forms are potentially life-threatening, particularly during infancy (Engel *et al.*, 2010).

A detailed study of five cases, started in collaboration with Ricardo Miledi and two of his visiting fellows, Stuart Cull-Candy and Alain Trautmann, found heterogeneity between the patients in terms of their miniature endplate potential amplitudes and AChR numbers (Supplementary material B39). Whereas four patients, including two brothers, had reduced AChRs, not dissimilar to those in many patients with myasthenia gravis, one patient had completely normal AChR numbers despite very small miniature endplate potential amplitudes [later identified by postdoc Richard

Webster as harbouring an AChR  $\alpha$  gene (*CHRNA1*) mutation causing abnormally small AChR channel openings]. JND, often with Peter Molenaar, continued to study muscle biopsies of patients with CMS, with Nick Murray at the National Hospital and Dennis Wray at the Royal Free Hospital, and later in Oxford with Kerry Mills at the Radcliffe Infirmary and Mark Roberts or postdoc Richard Webster doing the *in vitro* work. The neuromuscular junction defects were described in a large number of cases, in most of whom the molecular defects were eventually identified many years later.

In the 1990s, with the advent of the polymerase chain reaction and advances in sequencing techniques it became feasible to screen genes efficiently for mutations. As the genes encoding human proteins were cloned and the primary sequences determined (Supplementary materials B109 and B149; Buckle *et al.*, 1996), research assistant Rebecca Croxson and clinical fellow Phil Nicolls were able to identify mutations in *AChR* and *rapsyn* genes. The work really took off from then, with postdoc Claire Newland recruited to analyse the functional effects of the mutations. She obtained a detailed description of the consequences of the different genetic defects on AChR expression and function using patch and cell recordings from *Xenopus* oocytes, or from HEK (human embryonic kidney) 293 cells transfected with the mutant or wild-type genes. Novel  $\alpha$  subunit mutations were identified and characterized in the Slow Channel Syndrome (Supplementary material B190) and the adult AChR-specific  $\epsilon$  subunit gene was found to be a major target for CMS-associated mutations, probably, as suggested by Andrew Engel and colleagues, because loss of the  $\epsilon$  subunit could be partially compensated by maintaining expression of foetal AChR (that has the  $\gamma$  subunit instead of  $\epsilon$ ). The gene mutations were located throughout the length of the  $\epsilon$  subunit coding sequence, and along with the first identification of AChR promoter sequence mutations, were shown to cause reduced AChR expression and thus underlie AChR deficiency syndromes (Supplementary materials B216, B227 and B239).

However, AChR  $\epsilon$  mutations did not explain all of the patients with reduced endplate AChR expression, and mutations in *rapsyn*, the protein that anchors AChRs at the neuromuscular junction, were also identified (Ohno *et al.*, 2002). Between them these two gene targets were mutated in many patients with CMS and each was systematically characterized *in vitro* (Supplementary material B245; Ealing *et al.*, 2002; Cossins *et al.*, 2006). Georgina Burke, working in the clinic with JND, was able to identify different clinical phenotypes in these two forms of AChR deficiency with respect to ophthalmoplegia, arthrogryposis, squint and apnoeas, enabling targeted gene screening (Supplementary material B256).

Shortly before JND's retirement, Jackie Palace began to take over the clinical care of the patients, and JND helped to establish Oxford as the CMS hub of a National Commissioning Group (NHS) for rare neuromuscular disorders (with Kate Bushby in Newcastle and Mike Hanna and Francesco Muntoni in London), which he directed until 2007. The clinical and experimental work has flourished with the important identification of limb-girdle myasthenic syndrome associated with mutations in the recently-discovered *DOK7* gene, the clinical correlates (Supplementary materials B67 and B275), and the realisation

that treatment with ephedrine or other  $\beta$ -2 adrenergic receptor agonists such as salbutamol can lead to dramatic patient improvements in this now commonly recognized CMS (Lashley *et al.*, 2010). Indeed, a greater understanding of the genetics and molecular mechanisms underlying CMS means that treatments can now be tailored to the different forms, all of which show at least a partial response to the appropriate drugs. Thus, anti-cholinesterases, 3,4-diaminopyridine, AChR open-channel blockers or salbutamol/ephedrine may all be beneficial alone or in combination depending on the syndrome (see also Engel *et al.*, 2010).

Of course, the hope is as always for a more specific therapy. John Ealing, MRC clinical training fellow, joined the group to show that DNA hammerhead ribozymes could be used to repair or change AChR gene sequences (in collaboration with Professor Matthew Wood, Oxford), and had considerable success *in vitro*. But for further studies animal models have been created and studied electrophysiologically by postdocs Judith Cossins and Richard Webster for AChR deficiency (Cossins *et al.*, 2004) or the slow channel syndrome (Webster *et al.*, 2010), so that now potential drug or genetic therapies can be suitably trialled experimentally.

## Lambert Eaton myasthenic syndrome

One of JND's talents was to recognize an autoimmune disease on the basis of clinical hints—not unlike the autoimmune associations in patients with myasthenia and/or their relatives that first convinced Iain Simpson that myasthenia gravis was mediated by antibodies (Simpson, 1960). In the early 1980s, JND started to see patients with the Lambert Eaton myasthenic syndrome, which is often associated with small cell lung cancer. First characterized by Eaton and Lambert (1957), it was associated with a marked decrease in the presynaptic release of the acetylcholine packets (Elmqvist *et al.*, 1968; Lambert and Elmqvist, 1971). Indeed Nick Murray and JND had begun to treat patients with 4-aminopyridine, a drug that increases acetylcholine release but resulted quite frequently in seizures or other CNS disturbances (Supplementary material B35); with Jackie Palace, JND later popularized 3,4-diaminopyridine for both CMS and Lambert Eaton myasthenic syndrome.

Back then, the cause of Lambert Eaton myasthenic syndrome was unknown; paraneoplastic aetiologies were only just beginning to be studied in detail and some held cancer-related neurotoxic factors responsible for reducing transmitter release (Lambert and Lennon, 1982), although an immune basis was also suggested (Lennon *et al.*, 1982), and had been for the non-tumour cases (Gutmann *et al.*, 1972). On a speculative basis, JND plasma-exchanged three patients (Supplementary material B33). All showed relatively short-lived clinical improvements, which took 15–20 days to reach maximum (versus 3–5 days in myasthenia gravis). That encouraged him to start using prednisolone and azathioprine, achieving a remarkable improvement and successive increase in the muscle action potential in one patient (Supplementary material B33).

These successes clearly supported the idea that Lambert Eaton myasthenic syndrome was antibody-mediated, but the offending factor could still have been a biologically active peptide or other



circulating substance that was susceptible to immunosuppression. To justify the effort needed to identify the target and establish an antibody test, it was important to show that IgG antibodies were involved. Bethan Lang was cajoled into injecting 10 mg of purified Lambert Eaton myasthenic syndrome IgG intraperitoneally into mice every day (including weekends) for 37–77 days! The quantal content of neuromuscular transmission studied by Dennis Wray was significantly reduced in all test animals versus controls, but with no effect on miniature endplate potential amplitudes or AChR numbers (Supplementary material B33), strongly indicating that the presynaptic defects in patients were IgG-mediated.

These findings stimulated many studies in Lambert Eaton myasthenic syndrome; Peter Molenaar showed that acetylcholine contents of Lambert Eaton myasthenic syndrome muscle were normal, but confirmed that the release was deficient (Supplementary material B42), as also found in the mouse model (Supplementary material B60). And further passive transfer experiments detailed the reduction in quantal content at rest, and the lack of decrement (increments were rare in the mice) during repetitive trains at high frequencies (Supplementary material B48). In fact, the effects developed over the first few days, declined in parallel with falling human IgG levels after injections ceased, and were independent of mouse complement (Supplementary material B68). They clearly involved the voltage-gated calcium channel, since the calcium-dependency of AChR release was reduced at all calcium concentrations, and both nerve-evoked and high  $K^+$ -induced acetylcholine release were inhibited (Supplementary material B87). These experiments were paralleled by a very successful collaboration between JND and Dr Andrew Engel at the Mayo Clinic whereby mouse diaphragms were fixed and sent to Minnesota, USA for freeze fracture and electron microscopy. Engel and a succession of his visiting Japanese clinical fellows demonstrated beautifully the normal clustering of the presynaptic active zone particles that are voltage-gated calcium channels, and their disorganization/disappearance over time, indicating internalization and destruction of the protein—analogue to the antigenic modulation shown earlier in myasthenia gravis—which correlated with the electrophysiological defects seen in Oxford (Supplementary materials B47, B84, B85, B86, B104 and B110). To prove that this was also caused by divalent antibodies cross-linking the target protein (calcium channels assumed), monovalent Fab fragments did not achieve the same effect (Supplementary material B128). Meanwhile, an association with HLA DR3-B8 had been found in Lambert Eaton myasthenic syndrome (Supplementary material B76; later confirmed by Wirtz *et al.*, 2005), and Stephanie Robb and postdoc Terence Bowley had used the newly acquired and much prized Fluorescence Activated Cell Sorting machine to follow lymphocyte subsets during treatment (Supplementary material B70).

By the mid-1980s, the race was on to confirm that the antibodies were indeed directed against voltage-gated calcium channels and to establish an assay that could be used for diagnosis. Bethan Lang and her colleagues turned to studying cell lines that expressed the channels. A breakthrough was when medical student Victor Tse and Andy Roberts showed that the IgG from patients inhibited the  $K^+$ -induced calcium influx into cell lines derived from small cell lung cancers—the cancers most strongly associated with

Lambert Eaton myasthenic syndrome—indicating for the first time a direct relationship between the antibodies and these tumours, and published in *Nature* (Supplementary material B72). Indeed, small-cell lung cancer patients with Lambert Eaton myasthenic syndrome appear to survive better than those without the autoimmune disease as later shown by Paul Maddison (Supplementary material B223). The inhibition of calcium influx correlated with disease severity (Supplementary material B113); in the rodent neuroblastoma/glioma cell line NG108, it appeared to be targeting L-type calcium channels (Supplementary material B128). But it took the discovery of cone snail neurotoxins by Oliveira and colleagues (1987) to provide the tools needed for distinguishing the different calcium channel subtypes and identifying the true target for the Lambert Eaton myasthenic syndrome antibodies. In 1989, Sher and colleagues (1998) reported the first positive results of immunoprecipitation of  $^{125}I$ -conotoxin GVIA-labelled N-type voltage-gated calcium channels, demonstrating the antibodies in ~40% of patients with Lambert Eaton myasthenic syndrome; similar results were reported for 36 patients from Oxford (Supplementary material B131).

This was encouraging but evidence was accruing that another toxin was required, since the GVIA conotoxin did not inhibit calcium channels at the neuromuscular junction. It was not until 1995 that visiting clinical fellow Masakatsu Motomura showed that voltage-gated calcium channel antibodies are present in >85% of patients with Lambert Eaton myasthenic syndrome using the MVIIC conotoxin to label the appropriate (P/Q-type) voltage-gated calcium channel (Supplementary material B177 and Lennon *et al.*, 1995). These antibodies were found in paraneoplastic and other Lambert Eaton myasthenic syndrome patients and not in disease control groups, but also in a few with small cell lung cancer without evident Lambert Eaton myasthenic syndrome. Within patients, they correlated well with clinical state during treatments. It was still not altogether clear how specific the antibodies were for the P/Q-type (MVIIC-binding) voltage-gated calcium channels, or whether other antibodies were involved; several studies in collaboration with other groups looked further at antibody specificities in paraneoplastic and non-paraneoplastic patients (Supplementary materials B195, B214, B152, B171 and B175).

A seminal review of 50 cases (Supplementary material B106) by JND with John O'Neill and Nick Murray highlighted the autonomic dysfunction that is often evident, but not always appreciated, in these patients (and was probably the explanation for the urinary retention that Bethan Lang found in a few of her injected mice). A visiting Nuffield Fellow from Melbourne, Sally Waterman, undertook a detailed study of the calcium channels mediating transmitter release at autonomic post-ganglionic synapses; all types of voltage-gated calcium channel were involved, albeit to different extents and mainly during high frequency stimulation (which is relevant to *in vivo* function) (Waterman, 1996). When the mice were given Lambert Eaton myasthenic syndrome IgG, not only were the P-type channels selectively decreased but other voltage-gated calcium channels were upregulated, implying compensatory mechanisms (Supplementary material B201), as previously shown in myasthenia gravis. In collaboration with Eli Lilly, clinical fellow Ashwin Pinto explored the effects of Lambert Eaton

myasthenic syndrome IgG on different voltage-gated calcium channel subtypes, in stably transfected HEK cells, and found a marked reduction in P-type voltage-gated calcium channel, with little effect on any of the other subtypes examined. When the same IgG preparations were applied to cerebellar granular cells in culture, however, the electrophysiological responses became less dependent on P/Q-type voltage-gated calcium channels, with an increase in the contributions of other voltage-gated calcium channels (Supplementary material B213). Bethan Lang's further work confirmed a similar upregulation of calcium channel subtypes at the mouse neuromuscular junction after injection of Lambert Eaton myasthenic syndrome IgG (Giovannini *et al.*, 2002).

## Seronegative myasthenia gravis

It was clear from the earliest days that 10–20% of patients with generalized myasthenia gravis did not have detectable serum AChR antibodies by the radioimmunoprecipitation assay, as originally mentioned by Lindstrom *et al.* (1976). In fact symptoms were often rather severe, involving bulbar and respiratory muscles, but the patients usually responded extremely well to plasma exchange—which JND used almost as a 'diagnostic tool' for distinguishing autoimmune from potentially genetic disorders—although they were often relatively resistant to pyridostigmine or conventional immunotherapies. Thymectomy did not seem to be helpful, and their thymuses showed only mild thymic changes (Supplementary material B139). Stuart Mossman, a visiting clinical fellow, undertook the first passive transfer experiments, using IgG prepared from plasma exchange samples and demonstrating a defect in nerve-evoked muscle responses in the injected mice with little effect on AChR numbers (Supplementary material B79). This suggested that seronegative myasthenia gravis IgG might also be targeting the presynaptic terminals, which indeed showed mild but still unexplained abnormalities in some mice, as demonstrated later in more detailed *in vitro* studies by PhD student, Judith Burges, with Dennis Wray (Supplementary material B161). They found reduced quantal contents with some seronegative myasthenia gravis IgG preparations, as well as reduced miniature endplate potential amplitudes, and confirmed the minimal change in AChR numbers. Several studies over subsequent years aimed to identify an antibody and its target in these patients, but the results were not conclusive. Serum reduced AChR function in cultured cell lines, often within a few minutes. This effect was reversible when the antibody was washed out, and appeared to be IgM rather than IgG (Supplementary materials B140 and B159; Spreadbury *et al.*, 2004)—and yet the passive transfer experiments had been conducted with IgG not IgM!

The field needed a new approach, and in 2000, following a meeting in Mossbach, Germany, with Werner Hoch, an independent investigator at the Max Planck Institute for Neurobiology in Tübingen (who died suddenly in 2006), clinical fellow John McConville and Hoch quickly identified IgG antibodies to muscle specific kinase in many of the patients with seronegative myasthenia gravis who had previously responded to plasma exchange (Supplementary material B235). These antibodies were present in the plasmas that had been used for passive transfer experiments

(mainly from patients with severe bulbar myasthenia gravis), and did not correlate with the IgM-mediated reduction in AChR function (Plested *et al.*, 2002). In fact, IgG from patients with muscle specific kinase antibodies did not directly affect AChR function *in vitro*. The target and mechanisms involved in the latter phenomenon remain unclear—as does the exact mechanism of action of the muscle specific kinase antibodies, which is being explored in animal models (S. Viegas *et al.*, in preparation).

A fitting tribute to JND's policy of building a multidisciplinary team was when clinical fellow, Isabel Leite, used co-transfected rapsyn to cluster AChR naturally on the surface of human embryonic kidney cells; about half of the previous negative patients were clearly positive for binding to the AChR under these conditions, probably because the high density of AChRs allowed the antibodies to bind divalently. Interestingly, these patients had more thymic changes than those who did not show this reactivity (Leite *et al.*, 2008), suggesting that the aetiological and pathogenic mechanisms may be similar to those of typical AChR antibody-positive myasthenia gravis, a finding confirmed in animal models (S. Jacob *et al.*, in preparation).

## Peripheral neuropathies and acquired neuromyotonia

In the 1990s with experimental work on Lambert Eaton myasthenic syndrome beginning to slow down, JND enjoyed a brief sojourn among the gangliosides in collaboration with Professor Hugh Willison in Glasgow. They showed that the neuromuscular junction could also be used to study those antibodies (Supplementary materials B169 and B180), leading to much more detailed investigations by Willison and his collaborators, including Peter Molenaar's former colleague Jaap Plomp.

But JND's main new focus at this time was acquired neuromyotonia. This condition was probably first documented by A. Morvan as 9 of the 10 cases he described as 'chorée fibrillaire' did not have the CNS involvement that is now associated with 'Morvan's syndrome' (Morvan, 1890). Again it was the combination of occasional tumours (thymomas), other autoimmune associations, and a few reports of precipitation by penicillamine treatment, that drew JND's attention to a possible autoimmune aetiology (reviewed with Kerry Mills, Supplementary material B153). The first patient he saw with this condition was a retired Greek colonel (ANA, Supplementary material C) who responded to plasma exchange with a dramatic reduction in the typical neuromyotonic discharges recorded by Kerry Mills. Further clinical studies resulted and another antibody-mediated disease was proposed.

The laboratory work followed the now familiar pattern of passive transfer of IgG to mice to demonstrate that there were pathogenic antibodies. This was accomplished by a medical student Sourab Sinha (Supplementary material B136), and followed up later by visiting paediatrician Paul Shillito who investigated the neuromuscular junctions in more detail. Although *in vitro* results using neuronal cell lines were not conclusive, everything pointed to involvement of a potassium channel, and an immunoprecipitation assay was set up by PhD student Katherine Leys with radioactive dendrotoxin to label detergent extracts of brain

voltage-gated potassium channels. The procedure was analogous to that used for voltage-gated calcium channel antibodies in Lambert Eaton myasthenic syndrome, but was less successful, with only a proportion of plasma exchange-responsive patients proving to be positive with this test and usually with rather low titres, many not very different from the control range (Supplementary material B182). Nevertheless, Shillito's *in vitro* studies on five patients' IgGs, and those of Peter Molenaar and colleagues in Leiden, supported the idea that voltage-gated potassium channel antibodies were the pathogenic target (Supplementary material B182). But to increase sensitivity, another approach was needed, and MRC clinician scientist Ian Hart, who JND had recruited to Oxford after his PhD with Professor Martin Raff at UCL, developed an indirect immunohistochemical assay using *Xenopus* oocytes that he had injected with messenger RNAs for the different voltage-gated potassium channel subunits (Supplementary material B193). This proved somewhat more sensitive and showed that the antibodies were heterogeneous in their specificity for the different dendrotoxin-binding voltage-gated potassium channel subunits.

JND with Ian Hart (who died suddenly in 2008), Paul Maddison and Kerry Mills (who moved to a Chair at King's College) put together an impressive clinical and neurophysiological description of the disease in 67 patients with 'peripheral nerve hyperexcitability' (a more accurate description than 'neuromyotonia'), including patients with cramp fasciculation syndrome who shared similarities (Supplementary material B240). But the serology was always disappointing in patients with neuromyotonia. However, the high levels in a patient with Morvan's syndrome who made a substantial recovery with plasma exchange, and a chance conversation with Joel Oger (neurologist in Vancouver) led to the appreciation that such antibodies were also present in patients with CNS disease, including limbic encephalitis (Buckley *et al.*, 2001). Only in the last few years has it become clear that the antibodies are not mainly directed against the voltage-gated potassium channels themselves in these patients, but against other proteins that are complexed with the voltage-gated potassium channels in brain tissue (Irani *et al.*, 2010; Lai *et al.*, 2010).

## John Newsom-Davis as clinician

Although JND's excitement seemed to stem mostly from laboratory work, he did not neglect clinical studies and he encouraged the work of those young clinicians who were more suited to 'dry' rather than 'wet' research, particularly if they could lead to a better understanding of the diseases that he had helped to define.

In the early days at Queen Square, he was a major collaborator on Richard Hughes' highly cited trials of plasma exchange in Guillain-Barré syndrome (Supplementary materials B16 and B40), and involved in a number of significant case reports including rabies encephalitis (Supplementary material B12), one of the first cases of Creutzfeldt-Jakob disease after growth hormone (Supplementary material B67), and head injury in boxers (Supplementary material B7). From then, apart from a continued

interest in respiratory function in different diseases (Supplementary materials B24, B25, B114, B126 and B145), most of his focus was on neuromuscular junction disorders. While still at Queen Square, and later in Oxford, JND twice asked whether there were central cholinergic functional effects in myasthenia gravis or following treatment (Supplementary materials B115 and B186)—there weren't. But his most productive interactions were with the neurophysiologists. With Nick Murray he investigated the use of 4-aminopyridine and documented the response to plasma exchange in the Lambert Eaton syndrome (Supplementary materials B35 and B61). Then, as mentioned above, with John O'Neill, Murray and JND reviewed in detail the clinical and physiological aspects of 50 cases of Lambert Eaton myasthenic syndrome, which remains the most cited and comprehensive account of the disease (Supplementary material B106). Work on this condition was continued by JND's visiting fellows and registrars. Giles Elrington performed one of the first systematic studies of paraneoplastic disorders in patients with small cell lung cancer (Supplementary material B130), although Lambert Eaton myasthenic syndrome was not a common association (Supplementary material B284); Peter Bain showed the effectiveness of intravenous immunoglobulin in a cross-over study (Supplementary material B183); Colin Chalk demonstrated the response of Lambert Eaton myasthenic syndrome to treatment of the associated small cell lung cancer (Supplementary material B122); and Paul Maddison, conversely, showed a better outcome for the small cell lung cancer in patients with Lambert Eaton myasthenic syndrome (Supplementary material B236). Paul Maddison, with Kerry Mills, also detailed the neurophysiology of Lambert Eaton myasthenic syndrome (Supplementary materials B205, B206 and B207), and later of neuromyotonia (Supplementary materials B222 and B271) and Maddison and JND wrote two valuable Cochrane Reviews of treatment (Supplementary materials B248 and B263).

Jackie Palace, initially splitting her time between the laboratory and the clinic, conducted two important studies with JND—the effects of 3,4-diaminopyridine in congenital forms of myasthenia (Supplementary material B135) and the key trial of azathioprine as a steroid-sparing agent in myasthenia gravis (Supplementary material B212). The clinical features of patients with CMS that David Beeson identified, including many with *Dok-7* mutations, were characterized (Supplementary material B275), and Georgina Burke documented useful clinical clues to the diagnoses of AChR and rapsyn deficiencies (Supplementary materials B246 and B256). The discovery of muscle specific kinase antibodies provided opportunities for a new look at patients without AChR antibodies; Maria Farrugia working with Robin Kennett, JND and Professor Paul Matthews showed the distinctive electrophysiological and muscle imaging features of this disease (Supplementary materials B268, B269 and B273).

JND provided useful treatment algorithms for diseases he studied (e.g. Supplementary material B210). Use of treatments such as steroids, intravenous immunoglobulin and thymectomy provided not only food for thought but also some headaches. Steroids could cause myopathies (Supplementary material B154), intravenous immunoglobulin was useful but mechanisms were unclear and sometimes there were serious and unpredictable side-effects (Supplementary materials B156 and B244), and the



efficacy of thymectomy in myasthenia gravis had never been tested. After his retirement, despite a little more time with his family, JND switched focus to clinical trials in a characteristically effective manner, helping to promote better and more comprehensive reporting of their results (Supplementary material B242) and establishing with US colleagues Gil Wolfe, Henry Kaminski, Fred Jaretski and Gary Cutter, among others, the first blinded randomized clinical trial of thymectomy in myasthenia gravis which is still recruiting today (Supplementary materials B254, B276, B280 and H).

## Epilogue

The achievements of JND and the scientists and clinicians who worked with him over the 20 years from 1977 to 1998 when he retired, were focused on a small anatomical structure and a relatively rare set of neurological diseases, but they had substantial implications for the recognition and treatment of immune-mediated disorders as recognized by election to Fellowship of the Royal Society in 1991 (Fig. 4A). JND would have been the first to admit that being in the right place at the right time, and able to attract talented, hard-working and loyal people, were all crucial for his success. Having said that, his exceptional ability was to be fully involved with the laboratory research—indeed clearly happiest when, changed into his corduroys and a sweater, he was at his desk or chatting by a laboratory bench in the Institute of Molecular Medicine (now Weatherall Institute of Molecular Medicine). Moreover, unlike some in his position, he took co-authorship seriously, always making time to read drafts carefully, and making constructive suggestions—or indicating the need for reconstruction! He also had the integrity to opt out when he had not contributed substantially.

Over that time, he played a key part in both the clinical and research training of over 30 neurologists (see for instance Buckley; Supplementary material I), not only from Britain and its Commonwealth but also from Europe and the Far East. As Guy Chauplannaz from Lyons, France, wrote following JND's death, 'John was a truly European scientist. Dealing with rare diseases, he understood that co-operation with teams outside the UK could bring success sooner. He developed such friendly co-operations with warmth and efficacy'. Indeed, JND was loved and greatly respected by innumerable patients (Burrow; Supplementary material J). Many were especially grateful for his thoughtful and egalitarian attention when adapting their management to suit their particular needs; also for giving them the courage to keep fighting their myasthenia gravis—even though, as he often said, it would be 'a marathon and not a sprint'—and sometimes urging them to go ahead and have children, despite previous advice to the contrary (N. Willcox, personal communication and Supplementary material K) 'It is also impossible to overstate John's contributions to the evolution of medical care and research at Oxford in his role as a top-class clinical scientist and clinical neurologist. With his delightful, ever-youthful personality he was a wonderful colleague and much beloved by his students and all those who worked for him during his remarkable career' (Sir David Weatherall).

His sudden death while visiting Romania shocked neurologists throughout the world but his legacies—ongoing neuroimmunological and genetic research in Oxford, a generation of neurologists who acknowledge his many contributions to the discipline and to their own careers, the International Thymectomy Trial, and the rooms at the Academy of Medical Sciences that were recently named in his memory—are only a few of those that bear witness to his immense intellectual abilities and personal qualities. But even his closest colleagues did not appreciate the full extent of his contributions, both professional and personal until, following his death, the tributes and recollections began to flow in from patients and doctors all over the world.

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## Supplementary material

Supplementary material is available at *Brain* online.

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