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Neuroscience

Research in Neurophysiology began in 1951, with the appointment of John Eccles, the oldest (b. 1903) and most distinguished (FRS, 1941) of the founding professors, as Professor and Head of the Department of Physiology. He moved to the temporary laboratories in Canberra in 1953, and with the help of Jack Coombs, the electronics engineer who accompanied him from New Zealand, began work on neurotransmission for which he was awarded the Nobel Prize in 1963. From the outset, and especially after the move to the permanent building in 1957, he attracted a succession of distinguished Visiting Fellows and bright students. Among the latter was David Curtis, who graduated PhD in 1957, set up a subdepartment of Neuropharmacology in 1962, was appointed Professor in 1966 and, when space became available, Head of a new Department of Pharmacology in 1973. In 1967 Eccles was succeeded as Head of the Department of Physiology by Peter Bishop, who established a strong group in Visual Science. In 1984 he was succeeded by Peter Gage, but visual neuroscience was maintained by William Levick, who was appointed a Professor within the Department of Physiology in 1983. Studies in neuroscience were further expanded in 1980, when Robert Porter was appointed Director of the JCSMR. Bringing with him a large NH&MRC grant, he set up an Experimental Neurology Unit, of which Stephen Redman was appointed Head in 1984. With the introduction of the divisional structure in 1989 the groups headed by Curtis, Gage, Levick and Redman were combined as the Division of Neuroscience.

Life in Eccles' Laboratory

by David Curtis

Sir John Eccles (see p. 53), when reflecting in 1977 upon his peripatetic scientific career, wrote about his period in the JCSMR from 1952 until 1966: 'Without doubt it was the high point of my research career'. Similar words would be used by many of his close collaborators when referring to the time they spent in Canberra during the same period. When Eccles commenced his research career in 1927, with an investigation of cat spinal reflexes as a pupil of Sir Charles Sherrington in Oxford, there was

considerable speculation as to whether excitation and inhibition at mammalian central synapses were chemical or electrical processes. At that time, however, equipment and techniques were not available for investigating these processes at a cellular level *in vivo*. Accordingly, Eccles studied synaptic transmission in autonomic ganglia and neuromuscular junctions *in vitro*, initially in Oxford and later in Sydney from 1937 until 1943. His observations resulted in a firm belief that central synaptic trans-

mission was an electrical process.

From 1944 until 1951 Eccles occupied the Chair of Physiology in the University of Otago, and advances in neurophysiological equipment and techniques enabled him to resume his interest in the spinal cord *in vivo*. Results to 1950, including an analysis of potentials recorded extracellularly near spinal motoneurons using insulated metal electrodes, reinforced his opinion that central neurotransmission was electrical. In June 1951, however, he and his colleagues Jack Coombs (see p. 413) and Laurence Brock pioneered the extremely difficult task of recording intracellular potentials from spinal motoneurons *in vivo*, using electrolyte-filled glass microelectrodes. The initial results led Eccles to immediately and dramatically reject his very strongly held electrical theories and to accept that central synaptic transmission was chemical in nature. Further experimentation in Dunedin, however, was prevented by the requirement for further specialized equipment to be designed by Coombs, together with Eccles' acceptance of the JCSMR Chair and his commitment to deliver the Waynflete Lectures in Oxford early in 1952.

In 1950, Coombs, a physicist later aptly acknowledged by Eccles as a shy genius, began the design of an innovative, versatile, reliable and readily operated electronic stimulating and recording unit, which became widely known as the 'ESRU' (see p. 412). This unit, based on the advice of Eccles and Archie McIntyre, Reader in Eccles' Department, regarding the requirements of neurophysiologists, provided facilities which were unmatched in laboratories elsewhere. Coombs also designed stable and low-noise amplifiers and 'cathode-follower' input stages essential for recording through high resistance microelectrodes. Accompanying Eccles to Canberra in 1952, he established an electronics workshop in the Department and continued to develop a large range of specialised equipment, in addition to participating in the animal experimentation.

In the total absence of appropriate apparatus from commercial sources in Australia

and abroad, Eccles also insisted on having first class mechanical workshop facilities and personnel in his Department. Two remarkably innovative and skilled mechanical and electrical engineers were appointed as Head Technical Officers, Gerry Winsbury from 1952 to 1958 and Lionel Davies from 1959. They designed and constructed animal frames, micromanipulators (see p. 62), vertical microelectrode 'pullers' and numerous other specialised devices required for the technically difficult task of recording intracellularly from central neurons *in vivo*. Many items of equipment developed in the Department were later manufactured in the School Workshop, some even became available commercially with no benefit to the School, apart from an occasional 'Canberra-type' label. Eccles was also fortunate in bringing to Canberra Arthur Chapman, who had previously provided skilled technical assistance in his laboratories in Oxford, Sydney and Dunedin.

Thus, when laboratories became available in the temporary JCSMR buildings in March 1953, the stage was set for a remarkable and intense period of research which continued for over 13 years. During this time 74 investigators, including PhD Scholars, from 20 different countries, worked in the Department of Physiology, 41 of whom collaborated and published with Eccles. Initially only three electrophysiological laboratories were available, but from 1957 the additional space in the School's permanent building included six large laboratories with electrically shielded rooms, which enabled expansion of the research staff and an enhanced interest in neuropharmacology and neurochemistry.

Given Eccles' extensive knowledge of experimental neurophysiology, particularly of the spinal cord, his strong motivation to exploit intracellular recording techniques in both the cord and elsewhere in the central nervous system, so retaining the lead he had established over other laboratories, and the opportunity he had to attract experienced and beginning investigators to Canberra, the Department rapidly became an exciting and dynamic scientific environment. Initially

his investigations centred on the spinal cord, including its neuronal organization (see pp. 414 and 416) and the mechanisms of synaptic excitation and inhibition (see p. 411). From 1961 he concentrated on dorsal column and related thalamic nuclei, and from 1962 on the hippocampal and then the cerebellar cortex (see p. 422).

Eccles had the ability to combine experienced investigators and new recruits to form productive research teams. As team leader he was demanding, and expected all members to cooperate and contribute fully. Groups of 2-4 were essential to maintain continuity of effort during *in vivo* experiments lasting as long as 36 hours, generally twice weekly. These were preceded by the preparation and filling of glass microelectrodes, and followed by the task of measuring photographic records on ever increasing lengths of 35mm film, calculations using either a slide rule or logarithmic tables and then plotting the results on graph paper. Later, with increasing sophistication of equipment and the availability of calculating machines, the processing of results became faster and less tedious, but the majority of illustrations in departmental publications continued to be based on photographs of records from oscilloscopes.

Few animal experiments failed to provide new and publishable results. Eccles was a prolific writer; his bibliography lists 568 items, including 19 books of which he was sole author of 12. His name on a publication always indicated his active participation in all aspects of the investigation, and based on research in Canberra over the 1953 to 1966 period he authored or co-authored 196 publications, including 4 books. The total number of papers published by all other members of the Department of Physiology over the same period was approximately 224. The Department was well served by one Secretary, who typed from a variety of handwritten originals all drafts and final manuscripts using 'carbon-paper' to prepare multiple copies.

Friday afternoon departmental seminars were the rule each week, dealing with new results, drafts of papers being prepared for

publication and critical analyses of other investigator's papers. One aspect of the laboratory appreciated by all members of the staff and research students, in both the temporary and permanent buildings, was its close proximity to the Medical Library. This incorporated an accumulating collection of neurophysiological and related discipline journals, to become one of the best in the world for many years, with 24-hour availability every day of the year. In these early days, long before journals world-wide could be accessed via the Internet, the School's relative isolation from Europe and USA was reduced to some extent by the receipt of correspondence and unsolicited reprints from colleagues abroad long before the journals arrived in Canberra. Later some journals were received by air-mail, as was *Current Contents*. Eccles' deep interest in the Library was recognized in 1997 by naming it The Eccles Medical Sciences Library.

During long periods of continuous experimentation, late supper in Eccles' study, frequently involving several teams, provided an opportunity to discuss current progress and particularly for him to talk about his earlier experiences, his travels to scientific conferences, his impressions of other scientists and colleagues and his assessment of their scientific achievements. The combination of Eccles' experimental expertise, scientific ambition and stimulation, together with the satisfaction of contributing to interesting and frequently new scientific achievements, provided experiences never to be forgotten by his collaborators. Despite the remote location of Canberra, the reputation of Eccles and the quality of research being carried out provided numerous opportunities for the staff and PhD scholars to meet and attend lectures and seminars by numerous distinguished scientists from abroad.

Apart from its scientific aspects, life in his laboratory also has to be seen in relation to living in Canberra, then a relatively isolated city with a population of only 28,645 in 1953 and 96,032 in 1966 (approximately 308,400 in 1998). Fortunately, the Univer-

sity had an excellent housing scheme and provided accommodation for staff, research students and visitors, albeit often in distant suburbs amongst the Australian wild life, and University House, a few minutes walk from the JCSMR, was available for those

without children. Eccles and his wife were gracious hosts at weekend tennis parties on the family court and at regular country dancing sessions; he was an enthusiastic expert in both forms of exercise.

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Biophysical Properties of Motoneurons and Ionic Mechanism of Central Synaptic Transmission

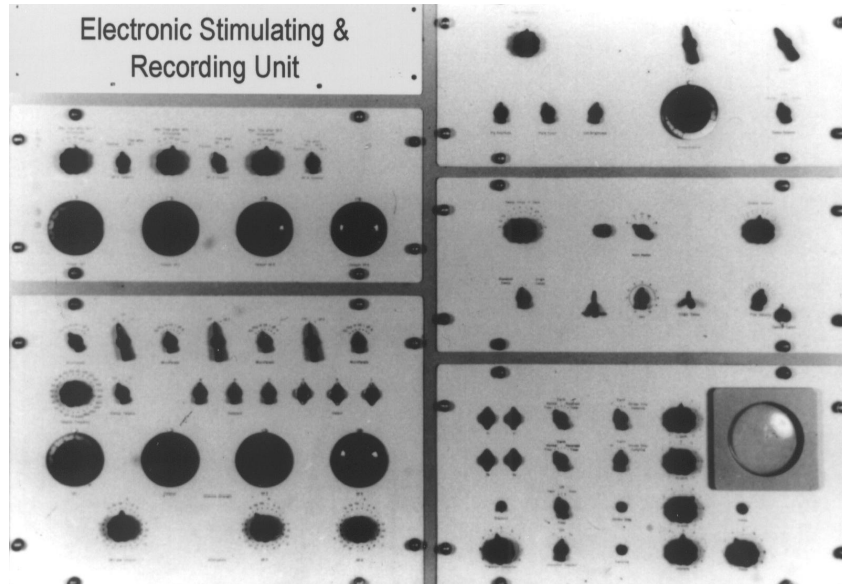
by David Curtis

In June 1951, John Eccles, Jack Coombs and Laurence Brock, working in Dunedin and recording intracellular potentials from cat spinal motoneurons *in vivo* for the first time, dramatically changed the understanding of the mechanism of synaptic transmission in the mammalian central nervous system. The recording of depolarizing excitatory and hyperpolarizing inhibitory post-synaptic potentials convinced Eccles that his previously strongly held electrical theory of synaptic excitation and inhibition was incorrect, and provided the basis for further studies of chemical synaptic transmission using intracellular recording techniques when he took up his appointment in Canberra (see p. 26).

Beginning in March 1953, Eccles, Coombs and Paul Fatt used single- and double-barrel electrolyte-filled microelectrodes to record intracellularly from alpha motoneurons in the lumbar spinal cord of

anaesthetised cats. Fatt, a biophysicist, had previously used intracellular microelectrodes with Bernard Katz at University College London to examine the changes in membrane ion permeability at excitatory synapses of the neuromuscular junction and at inhibitory synapses on crustacean muscle. In the spinal cord, one barrel of double-barrel electrodes was used to record membrane, action and synaptic potentials. The other enabled current to be passed through the neurone membrane to set the membrane potential above or below the resting level. Additionally, ions could be ejected from single, or either barrel of double, electrodes by suitably directed electrical currents in order to alter intracellular cation or anion levels.

The electrical properties of the motoneurone membrane were measured, and the characteristics studied of the ion fluxes and pumps contributing to resting and action



The Electronic Stimulating and Recording Unit (ESRU) was designed by Jack Coombs while he was in the Physiology Department of the University of Otago. It was based on the use of thermionic 'valves', and a double-beam oscilloscope having a linear time base and the facility for sweep expansion. In 1951 this provided unique facilities for neurophysiological research. Three electrical pulses, independently controlled for intensity, duration and timing along the oscilloscope sweep, were available to stimulate, via isolation transformers, the same or different nerves. The prototype unit, constructed in Dunedin, was later used by Eccles in Canberra from 1953 until 1966. The Industrial Development Department of Canterbury University College in Christchurch undertook the mechanical design and construction of four units for the Department of Physiology of JCSMR; two were installed in the Department's temporary quarters in 1951–52, and all four in the new School building in 1957. The stimulator portion of one was in continuous use in the Department of Pharmacology until late 1985, and provided the basis for the design and manufacture by A. Saeck of an entirely solid state stimulating unit which was used until 1995.

potentials. The observation that inhibitory postsynaptic potentials, initially recorded as hyperpolarizations, gradually converted to depolarizations, led to the recognition that this was the consequence of the leakage of ions from intracellular recording microelectrodes containing potassium chloride. Electrodes were then used containing combinations of a series of cations and anions of different hydrated ion diameter, from which ions were passed intracellularly. This technique was combined with an examination of the influence of changes in membrane resting potential on inhibitory and excitatory synaptic potentials.

The results indicated that at the synapses on motoneurons of the 'direct' and recurrent spinal inhibitory pathways the activated postsynaptic membrane was transiently

converted to a sieve-like structure permeable to ions smaller than a critical size. An increased permeability to chloride and potassium ions thus accounted for hyperpolarizing membrane currents and potentials. In contrast, depolarization at excitatory synapses in the spinal cord was generated by a non-selective increase in the permeability to larger ions including sodium, so transiently short-circuiting the membrane potential. It was essentially these and later related studies which resulted in the award of the Nobel Prize to Eccles in 1963.

Coombs, Eccles and Fatt also examined the interaction between monosynaptic excitatory and 'direct' inhibitory potentials in terms of the transient nature of the change of permeability at inhibitory synapses and the time courses of the excitatory and



Left John Saxon Coombs (1917–93) received his BSc and MSc degrees from the University of Dunedin and was appointed to a Lectureship in the Department of Physics in that University. His involvement in neurophysiological research began in 1950 when asked by J.C. Eccles, then Professor of Physiology in the University, to design and supervise the construction of electronic stimulating and recording equipment having features which were then not provided by commercially available equipment. The highly successful production of the 'ESRU', and his close and continuing participation in the pioneering intracellular recording from spinal motoneurons, led to his appointment as a Fellow in the JCSPMR Department of Physiology in September 1952 (Electronics Engineer, 1961). In Canberra, from 1952 until his retirement in 1977, he continued to design and make innovative electronic equipment which provided special features of inestimable value to investigators in the Departments of Physiology and Pharmacology.

Right Masao Ito (1928–). Graduating MD (1958) and PhD (1959) from the University of Tokyo, Ito was an ANU PhD Scholar from 1959 until 1961, and a Research Fellow until May 1962. He investigated, with Eccles and a number of colleagues, the biophysical properties of motoneurons and the ionic conductance changes underlying synaptic inhibition in the spinal cord. On his return to the Faculty of Medicine, University of Tokyo, where he was successively Associate Professor (1963), Professor of Physiology (1970), and Dean (1986), he and his colleagues demonstrated that cerebellar Purkinje cells were inhibitory neurones, and proposed that the transmitter released was GABA. From 1989 he was a team leader, and from 1992, the Director General of the RIKEN Frontier Research Program. In 1997 he was appointed the Director of the newly established RIKEN Brain Science Institute. Amongst his numerous awards are Foreign Membership of the Royal Society (1992) and the Japan Prize (1996).

inhibitory postsynaptic potentials. The time courses of the transient ionic currents at inhibitory and excitatory synapses were estimated in 1955 by Eccles, Coombs and David Curtis (see p. 75) using directly measured electrical properties of the motoneuron membrane. Subsequent re-examination between 1959 and 1962 of the ionic processes contributing to the resting, action and synaptic potentials of motoneurons by Eccles and a number of colleagues,

including Masao Ito, using an extended series of anions and cations, essentially confirmed Eccles' earlier proposals regarding the involvement of chloride and potassium ions at activated spinal inhibitory synapses.

An investigation in 1955 by Eccles, Coombs and Curtis led to the description of the components of orthodromic, antidromic and direct action potentials of alpha-motoneurons in terms of the morphology of these cells. In 1956 Eccles, Rosamond

Eccles (see p. 415) and Anders Lundberg (see p. 416) demonstrated that there are two subclasses of spinal motoneurone, having axon conduction velocities and durations of post-action potential hyperpolarizations appropriate to the innervation of fast or slow extensor muscles. Subsequently, in 1958 Eccles, Rosamond Eccles and Arthur Buller showed by section and cross union of the

nerves to fast and slow muscles that the type of motoneurone innervating a muscle determined its contractile properties. Later studies from 1960 by Russell Close were concerned with the neural influence on the excitation-contraction properties which control the speed of shortening of fast and slow muscle fibres.

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Organization of Spinal Excitatory and Inhibitory Pathways

by David Curtis

Two very significant investigations which began in the Department of Physiology in 1953 indicated that central neurones had either an excitatory or an inhibitory effect on other neurones. John Eccles, Paul Fatt and K. Koketsu investigated the recurrent inhibition of motoneurones by antidromic volleys in the axons of other motoneurones. This postsynaptic hyperpolarization, which was reduced by the convulsant alkaloid strychnine, was found to involve the prior excitation of ventral horn inhibitory interneurons by acetylcholine at synapses of the intraspinal collateral branches of the axons of motoneurones. The inhibitory pathway was thus disynaptic, and the interneurons were called Renshaw cells in honour of Birdsey Renshaw (1911–1948) who had earlier recorded their action potentials at the Rockefeller Institute, New York.

This was the first direct demonstration that acetylcholine was a neurotransmitter in

the mammalian central nervous system. Furthermore, since the axons of motoneurons also release acetylcholine peripherally at the neuromuscular junction, this study also provided support for the proposal in 1935 by Sir Henry Dale (1875–1968) that the same transmitter is released at all of the synapses made by the axon of a neurone. Studies of the electrophysiology of Renshaw cells and of the distribution of recurrent inhibition in the lumbar spinal cord were subsequently carried out by Eccles, Rosamond Eccles, Ainsley Iggo, Masao Ito (see p. 413) and Anders Lundberg.

In the initial study by Eccles, Fatt and Koketsu some compounds effective at the nicotinic receptors at the neuromuscular junction had no action at the synapses between motor axon collaterals and Renshaw cells when administered systemically. When these compounds were administered microelectroretically (see p. 425) close to these

cells, however, David Curtis and Rosamond Eccles demonstrated in 1957 that a blood-brain-barrier accounted for these apparent anomalies. A further microelectrophoretic investigation of acetylcholine receptors on Renshaw cells by Curtis and Ronald Ryall from 1962 indicated the presence of nicotinic and muscarinic sub-types, both of which were probably involved in the excitation of these neurones by impulses in axon collateral fibres under experimental conditions.

Also in 1953, Eccles, Fatt and Sven Landgren showed that another strychnine-sensitive inhibition in the spinal cord, the 'direct' short-latency and short-duration hyperpolarizing inhibition of spinal motoneurons by afferent volleys, regarded at the time to be monosynaptic, involved the prior excitation of an inhibitory interneurone located in the spinal intermediate nucleus, and was thus disynaptic. Confirmatory evidence was later provided by Eccles, Ito and T. Araki in 1960. Accord-

ingly, the central synapses of primary afferent (dorsal root) fibres were established as excitatory, and the inhibitory effects of impulses in such fibres were accepted as following the excitation of relatively short axon inhibitory interneurons. In later studies, Eccles and his collaborators identified and established the role of other inhibitory interneurons in the spinal cord, dorsal column nuclei, thalamus, hippocampus and cerebellum (see p. 422).

An extensive study of the patterns of distribution of the excitation and inhibition of individual spinal neurones, including cells of origin of ascending pathways, by impulses in various types of primary afferent fibre of muscle and cutaneous origin commenced in 1956, initially involving Eccles, Rosamond Eccles and Lundberg, and later numerous collaborators, including Olaf Oscarsson, who had a particular interest in spino-cerebellar pathways.

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Rosamond Margaret (Eccles) Mason (1929–). After graduating MSc at the University of Otago in Dunedin, New Zealand, in 1951, Rosamond Eccles was an ANU Overseas PhD Scholar at the University of Cambridge and joined the Department of Physiology in Canberra in 1954 to complete her PhD thesis research. As a Research Fellow from 1955, Fellow 1962, she continued her investigation of transmission through mammalian sympathetic ganglia, and with a number of colleagues studied interneurons, including Renshaw cells, and motoneurons involved in spinal reflexes. With J.C. Eccles and A.J. Buller she studied the properties of motoneurons innervating fast and slow limb muscles, and later contributed to the investigation of presynaptic inhibition in the spinal cord. For family reasons she resigned in 1966, but maintained her research interests as a Visiting Fellow until 1968. Photograph by David Coward.

Anders Lundberg (1920–). Before graduating MD at the University of Lund in 1947 and PhD in Medicine at the University of Stockholm in 1948, Anders Lundberg started his research career in Lund in 1945, and moved to Stockholm in 1946 to work with Professor Ragnar Granit. He was a Visiting Investigator at the Rockefeller Institute, New York, from 1949 to 1950, and a Senior Research Fellow in the Department of Physiology, JCSMR, from January 1956 until October 1957. Prior to his period in Canberra he had used intracellular microelectrodes to investigate salivary glands, transmission in sympathetic ganglia and in the dorsal spinocerebellar tract. Returning to Sweden, he was Associate Professor of Physiology in Lund 1959–1961, Professor of Physiology in Goteborg 1961–86, and since 1987 has been Professor Emeritus in Goteborg. In Sweden, Lundberg and his colleagues extensively investigated the functional organization of afferent, ascending and descending pathways and interneurons associated with the reflex activity of the mammalian spinal cord.



Presynaptic Inhibition and Transmission in the Somatosensory Pathway

by David Curtis

In the 1950s, John Eccles and his colleagues showed that increases in membrane potential (hyperpolarization) and conductance accounted for the synaptic inhibition of spinal neurones (see p. 412). Inhibition in the mammalian central nervous system then became regarded as essentially a postsynaptic phenomenon, by which transmitters released at inhibitory synapses reduced the excitability of neurones. In 1957, however, Kay Frank and Michael Fuortes at the US National Institutes of Health provided evidence for another type of spinal inhibition, in which a prolonged reduction of excitatory transmission at synapses of primary afferent (sensory) fibres on motoneurones occurred in the absence of detectable changes in motoneurone membrane potential and

excitability. One explanation of this inhibitory process was a presynaptic reduction of the release of excitatory transmitter, 'presynaptic' inhibition.

From early 1960 until 1966 many members of the Department of Physiology, led by Eccles, became engaged upon an intensive investigation of the organization and mechanism of this type of inhibition in the cat lumbar and cervical spinal cord and dorsal column nuclei. The long latency and prolonged inhibition of monosynaptic spinal reflexes by stimulating segmental afferent fibres was found to be accompanied by depolarization of the excitatory terminals of the afferent fibres generating the reflex. This primary afferent depolarization was referred to as 'PAD'. Since changes in the

excitability of the postsynaptic neurones could not be detected, the inhibition appeared to be essentially presynaptic in nature, and was considered to be an important negative feed-back control mechanism of the relay of somatosensory information into the spinal cord and to supraspinal centres.

The types of muscle and cutaneous afferent fibre receiving and generating PAD were identified, as were the neuronal pathways associated with both the production of PAD, and the inhibition of spinal reflexes and of the relay of sensory information to supraspinal centres. A number of studies concerned the mechanism by which PAD depressed transmitter release. In 1961, Eccles, Per Andersen and Thomas Sears demonstrated that PAD was generated by impulses in pathways descending from higher centres of the nervous system. In addition to its role in the lumbar and cervical spinal cord, presynaptic inhibition was also shown to control the transmission of impulses in primary afferent dorsal column fibres through the dorsal column nuclei but not to be significant at the next relay in the somatosensory pathway through the ventrobasal thalamus.

Unlike postsynaptic inhibition in the spinal cord (see p. 412), presynaptic inhibition of spinal reflexes and PAD were not reduced by the convulsant alkaloid strychnine. Both the inhibition and PAD, however, were found in 1961 by Eccles, Robert Schmidt and William Willis to be reduced by intravenous picrotoxin. Picrotoxin, a convulsant without effect on strychnine-sensitive inhibition, was known to block the inhibitory effect of gamma-aminobutyric acid (GABA) in crustacea. Since this amino acid had been detected by other investigators in the mammalian brain and spinal cord in the mid-1950s, Eccles, Schmidt and Willis suggested that GABA could be the depolarizing transmitter generating PAD in the mammalian spinal cord.

Eccles had proposed in 1961 that PAD resulted from the prolonged action of a depolarizing transmitter at synaptic contacts (axoaxonic synapses) on the terminal bou-

tons of primary afferent fibres. The reduced release of transmitter from these boutons was attributed to a reduction of the amplitude of presynaptic action potentials, resulting from both the depolarization and the underlying increased membrane conductance. In 1962, George Gray reported in *Nature* that axo-axonic synapses occurred in the mammalian spinal cord, and later studies by others established that the postsynaptic elements of these complexes were the terminals of primary afferent fibres. The presynaptic elements, terminals of spinal interneurons, were also later shown to synapse upon neuronal dendrites in a 'triad' arrangement. The presence of these triads provided morphological support for several subsequent reports that 'presynaptic' inhibition in the spinal cord was often accompanied by postsynaptic hyperpolarization of motoneurons at predominantly dendritic synapses. Histochemical evidence also became available that GABA was most likely the transmitter synthesised and stored within the presynaptic terminals of these axo-axonic/axo-dendritic triads.

In 1967, David Curtis and his colleagues demonstrated that GABA hyperpolarized spinal motoneurons, an action not blocked by strychnine. Subsequently, in 1970, the role of this amino acid in spinal inhibition was supported by the finding of Curtis, Graham Johnston and their colleagues that both PAD and presynaptic inhibition of spinal reflexes were blocked by intravenous bicuculline. This convulsant alkaloid, a specific antagonist of central GABA receptors now recognized as being of the GABA-A subtype, also blocked the hyperpolarization of central neurones by GABA (see p. 429).

It was not until 1976, however, that Curtis, with David Lodge and later other colleagues in the Department of Pharmacology, combined microelectrophoretic (see p. 425) and electro-physiological techniques to investigate *in vivo* the pharmacological and biophysical properties of single primary afferent axons within the spinal cord. Direct evidence was obtained for the first time that GABA depolarized unmyelinated 'terminations' (presynaptic axons and synaptic bou-

tons) of primary afferent fibres, but had no effect on intraspinal myelinated axons. Depolarization by GABA was accompanied by an increased membrane conductance, and was blocked by microelectrophoretic bicuculline, which also reduced synaptically generated PAD of terminations.

The actions of a series of GABA analogues on afferent terminations indicated that the bicuculline-sensitive receptors were similar to those at which GABA hyperpolarized spinal and other central neurones by increasing the permeability of the postsynaptic membrane at inhibitory synapses to chloride ions. The higher intracellular concentration of chloride in primary afferent neurones, and their central terminals, than in neurones having cell bodies within the central nervous system accounts for the opposite actions of GABA on the membrane potentials of these cells. Both a conductance increase and depolarization of afferent terminal decreased the amplitude of presynaptic action potentials, so reducing the influx of calcium ions through presynaptic voltage-activated channels which initiates transmitter release. The effect of small depolarization alone is probably minimal.

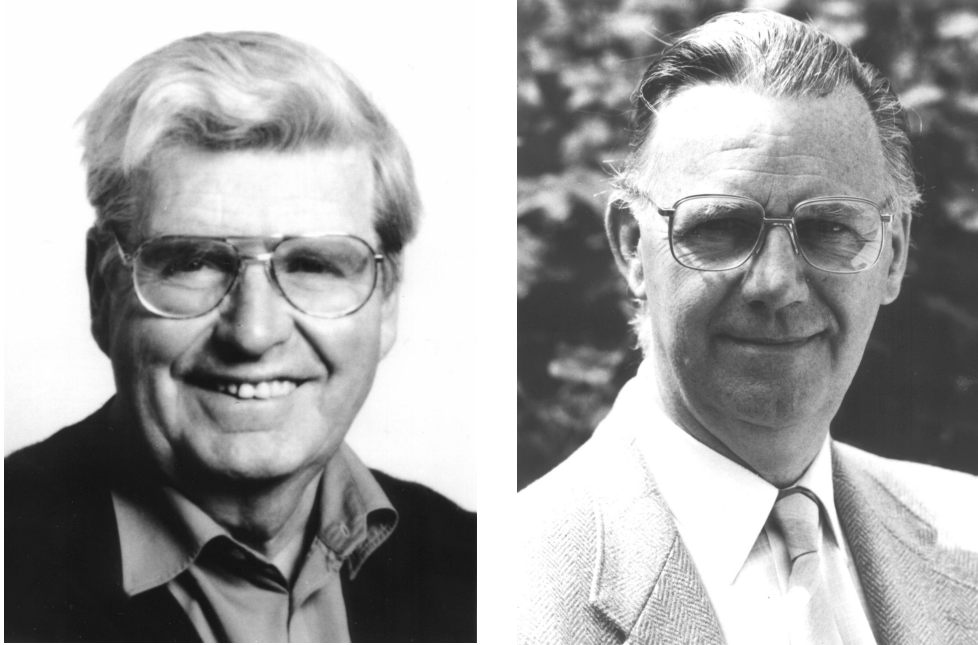
More recent investigations suggest that the role of GABA in spinal presynaptic inhibition may be more complex than activation of pre- and post-synaptic GABA-A receptors linked to chloride channels. In 1979, baclofen [β -(4-chlorophenyl)GABA], which had been synthesised by CIBA in 1962 as a potentially therapeutically effective GABA analogue which penetrated the

blood-brain barrier, was shown by Curtis, Lodge and their colleagues to selectively reduce the release of excitatory transmitter from primary afferent terminations in the mammalian spinal cord, an effect, however, not accompanied by depolarization and not blocked by bicuculline. In laboratories abroad, using tissue preparations in which the action of baclofen could be directly compared with that of GABA in the presence of bicuculline, both baclofen and GABA had been demonstrated to activate a bicuculline-insensitive receptor, now referred to as the GABA-B sub-type. Later, in 1988, a study in Canberra indicated that a reduction of the influx of calcium ions through channels activated by presynaptic action potentials was the most likely explanation of the reduction by baclofen of transmitter release at primary afferent terminations.

Subsequently, in 1992, following the synthesis by CIBA-GEIGY of selective GABA-B receptor antagonists which pass the blood-brain barrier, Curtis and Gary Lacey found that these antagonists considerably attenuated the long latency and prolonged 'presynaptic' inhibition of spinal reflexes. In contrast to bicuculline, however, these compounds were not convulsants and enhanced rather than reduced PAD. These observations raised as yet unanswered questions about both the role of PAD in the presynaptic inhibitory process and the functional significance of 'presynaptic' inhibition in the spinal cord.

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Left Per Oskar Andersen (1930–) graduated MD from the University of Oslo in 1954, PhD in 1960. He held academic appointments in the Anatomical Institute of the University from 1951 until 1964, and from November 1961 spent two years as a Rockefeller Visiting Fellow working with John Eccles and his colleagues studying presynaptic inhibition, synaptic mechanisms in dorsal column and thalamic nuclei and basket cell inhibition in the hippocampal and cerebellar cortices. From 1964 he was a member of the staff of the Neurophysiological Institute in Oslo, Associate Professor 1964–1972, Professor 1972–1997, and since 1997 has been Senior Stipendiat in the Physiological Institute. His major research interests include hippocampal neurobiology, the relation between structure and function of central neurones, and the synaptic mechanisms associated with learning and memory. One of Europe's most distinguished neuroscientists, he is a Member of a number of Academies, including the Norwegian Academy of Science, Academia Europea, and the Royal Swedish Academy, and is a Foreign Associate of the US National Academy of Sciences.

Right Thomas A. Sears (1928–). From 1961–1963 Sears, a Lecturer in the Electroencephalography Department at the Institute of Neurology, London, was a Wellcome Fellow in the Department of Physiology JCSMR. In addition to pursuing his interests in the electrophysiology and connections of thoracic respiratory neurones using intracellular recording techniques, he also worked with John Eccles and Per Andersen on presynaptic inhibition in the spinal cord evoked by stimulating the cerebral cortex and on transmission through ventrobasal thalamic nuclei. This latter study led to the development of a theory to account for the phased discharge of cortical neurones which underlies the generation of the electroencephalogram. His ANU PhD was awarded in 1964. Returning to the Institute of Neurology he continued to investigate the respiratory system and also undertook studies in experimental demyelination. In 1973 he was appointed a Professor at the Institute, and in 1975 Head of the newly established Sobell Department of Neurophysiology, a position he held until retirement in 1993. As Professor Emeritus he is currently an Honorary Senior Research Fellow at King's College London.



Left Robert Franz Schmidt (1932–) graduated MD from the University of Heidelberg in 1959 and was a PhD Scholar with John Eccles from 1960 to 1962 investigating presynaptic inhibition in the spinal cord. His ANU PhD was awarded in 1963, and following academic positions in the Institute of Physiology in Heidelberg he was appointed as Associate Professor in 1970. From 1971 until 1982 he was Professor and Director of the Institute of Physiology at the University of Kiel. Subsequently, from 1982 until 2000 he was Professor and Director of the Institute of Physiology at the University of Wurzburg, and is currently a Professor Emeritus of that University and an Associate Investigator at the Institute of Neuroscience, Univerisdad Miguel Hernandez, San Juan de Alicante in Spain. His research interests have continued to be concerned primarily with sensory neurophysiology, and his contributions have been recognized by the award of Lectureships and Honorary Memberships by many learned societies. In 1996 he received a DSc *honoris causa* from the University of New South Wales.

Right William Darrell Willis, Jr. (1934–) graduated MD from the University of Texas Southwestern Medical School, Dallas in 1960, and his ANU PhD was awarded in 1963 on the basis of research related to presynaptic inhibition carried out with John Eccles from 1960 until 1962. After a postdoctoral year in Pisa he returned to Dallas and was appointed Professor and Chairman of the Department of Anatomy from 1964–1970. He then moved to the University of Texas Medical Branch in Galveston as Chief, Laboratory of Comparative Neurobiology of the Marine Biological Institute, and has been Director of that Institute since 1978. Since 1986 he has also been Chairman of Anatomy and Neurosciences in Galveston. He has continued a research interest in spinal presynaptic inhibition, central pain pathways and the processing of nociceptive information.



Left David Lodge (1941–) graduated BVSc from Bristol University in 1963, and after seven year's involvement in veterinary clinical practice at the University's Veterinary Hospital, undertook PhD studies in the Department of Physiology, University of Bristol, graduating in 1974. In Canberra, from 1974–75 as a Postdoctoral Fellow and 1975–79 as a Research Fellow, he collaborated with David Curtis in a number of projects concerned with the central pharmacology of inhibitory and excitatory amino acids, including the role of GABA in spinal presynaptic inhibition. From 1979–91 he was successively Senior Lecturer, Reader, Professor and Head of the Department of Physiology at the Royal Veterinary College, London, investigating central excitatory amino acid receptors. In 1991 he joined Eli Lilly and Co as a Research Scientist, and is currently Research Advisor and Director of that company's Stroke Research Program.

Right Gary Lacey (1960–) graduated BSc(Hons) at Chelsea College, University of London, in 1983, and gained a PhD degree in Pharmacology at the Royal Free Hospital School of Medicine, London, in 1986. He then spent two years at St Bartholomew's Hospital Medical College, and was a Postdoctoral Fellow in the Division of Neuroscience, JCSMR, in 1989, and a Research Fellow from 1992 until his resignation in 1995. In the JCSMR he collaborated with David Curtis in investigating transmitter release mechanisms in the mammalian spinal cord, including the mode of action of baclofen and the role of GABA-B receptors in presynaptic inhibition. Since 1996 he has been a member of the Drug Safety Evaluation Branch of the Therapeutic Goods Administration in Canberra.

Excitation and Inhibition in Hippocampal and Cerebellar Cortices

by David Curtis

Per Andersen's (see p. 419) previous experience in Oslo of investigating excitatory pathways in the hippocampal cortex led in 1962 to a study with John Eccles and Yngve Løyning of the large and prolonged chloride-dependent inhibitory hyper-polarizations recorded from pyramidal cells in the CA3 region. Intracellular records from pyramidal cells, and extracellular records of the action potentials of interneurons, were combined with a systematic depth analysis of extracellular potentials generated by antidromic and afferent volleys. This latter analysis took advantage of the laminar arrangement of synapses originating from various sources. The study resulted in the identification of basket cells, the axon terminals of which form a dense network around the soma of pyramidal cells, as inhibitory interneurons. Such a location of inhibitory action would be the most effective site for controlling the discharge of axonal impulses by pyramidal cells. Subsequently, this very significant first correlation between the inhibitory function of a neurone and the arrangement and location of its axonal terminations served as a guide for the identification of other central inhibitory neurones and their synapses.

The following year, Andersen, Eccles and Paul Voorhoeve, using the same procedure, demonstrated that basket cells in the cerebellar cortex, which also has a laminar arrangement of synapses and neurones, inhibited Purkinje cells through synapses located on the soma. This was followed by an intensive and systematic study, led by Eccles and including Rodolfo Llinas, Kazuo Sasaki and Piergeorgio Strata, of the organization and properties of all major types of neurone in the cat cerebellar cortex, including their connectivity with other regions of the central nervous system and particularly by spino-cerebellar and olivo-cerebellar tracts. In this investigation, Eccles was

influenced by the Hungarian neuroanatomist, Janos Szentagothai, whose detailed knowledge of cerebellar microanatomy was unrivalled, and by the observations of Masao Ito (see p. 413) who had spent three years in Canberra from 1959. Later, in Tokyo, Ito and his colleagues had shown that Purkinje cells were inhibitory neurones, and, on the basis of antagonism by intravenous picrotoxin, proposed that GABA was probably the inhibitory transmitter at synapses made by Purkinje cell axons.

The Canberra group established that in addition to Purkinje and basket cells, superficial stellate and Golgi cells were also inhibitory neurones. Stellate cells inhibited Purkinje cells, and Golgi cells had a strong inhibitory action at mossy fibre-granule cell synapses of spino-cerebellar tracts to the cerebellar cortex. In contrast, granule cells and neurones of the inferior olivary nucleus were excitatory neurones, giving rise to parallel and climbing fibres respectively, both exciting Purkinje cells. The considerable convergence of hindlimb muscle, joint and cutaneous afferent impulses on olivary neurones was also demonstrated. This study of the mammalian cerebellum was continued by Eccles in the United States from 1967 until his retirement in 1975, and overall, as the first comprehensive analysis of the mode of operation of the main neurones of the cerebellar cortex, was a major contribution to the understanding of its functional role.

The proposal in 1961 by Eccles, Schmidt and Willis that GABA was likely to be the transmitter of strychnine-insensitive prolonged presynaptic inhibition in the spinal cord (see p. 417) led in 1963 to several investigations in the Department of Physiology of the effects of strychnine on the prolonged postsynaptic hyperpolarizing inhibitions of neurones in the hippocampal, cerebellar and cerebral cortices and in the thalamus. All of these supraspinal inhibi-

tions were found to be insensitive to strychnine, suggesting that either the transmitters or the postsynaptic receptors involved differed from those at strychnine-sensitive inhibitory synapses in the spinal cord. On the basis of antagonism by bicuculline, Curtis, Graham Johnston and their colleagues

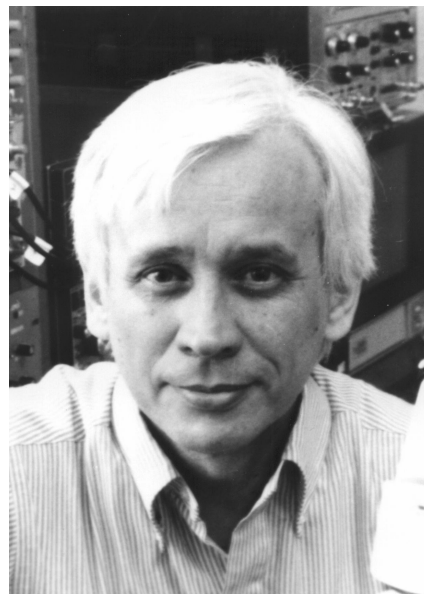
proposed in 1970 that GABA was the transmitter of supraspinal inhibitions not influenced by strychnine (see p. 429). These included basket cell inhibition of hippocampal pyramidal cells, Purkinje cell inhibition of lateral vestibular nucleus neurones and basket cell inhibition of Purkinje cells.

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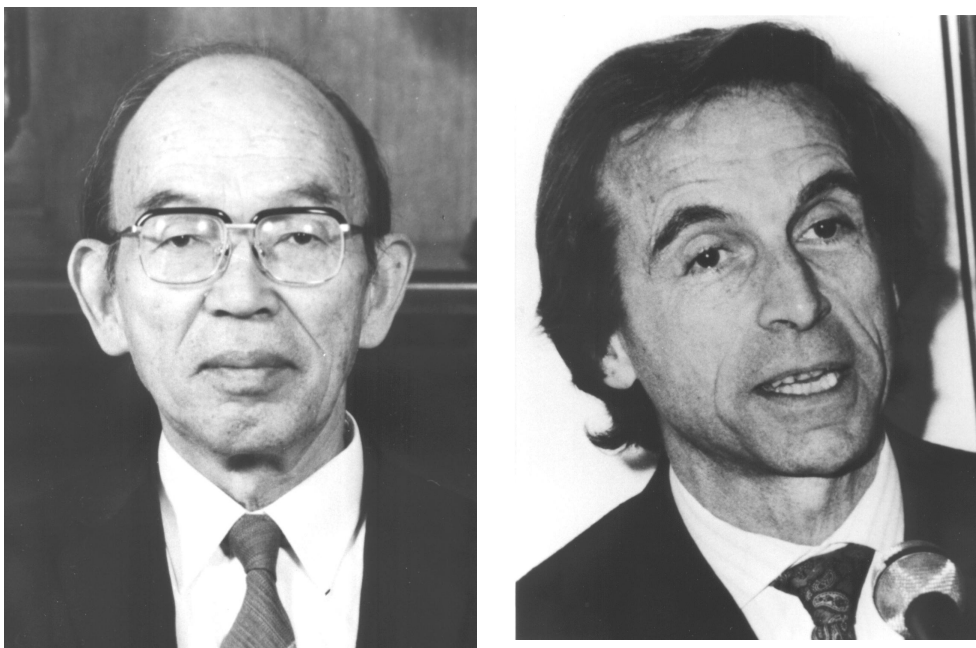
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Left Paul E. Voorhoeve (1927–) studied medicine and histology at the University of Amsterdam and physiology at the University of Leiden (The Netherlands), graduating MD in 1954. After two year's army medical service he carried out electro-physiological research on fusimotor neurones with Professor Yves Laporte at Toulouse and received his PhD in 1959. He then spent two postdoctoral years with Professor Anders Lundberg in Lund, and was a Visiting Fellow in the Department of Physiology, JCSMR from 1962 to 1964 collaborating with John Eccles on the cellular physiology of the cerebellar cortex. From 1965–1990 he was Professor of Neurophysiology at the University of Amsterdam.

Right Rodolfo Llinas received his MD from Universidad Javeriana in Bogota, Colombia in 1959, and was a Research Fellow in the Department of Neurosurgery, Massachusetts General Hospital, Harvard Medical School (1960–61), and in the University of Minnesota (1961–63). From 1963, as an ANU Research Scholar, he carried out research with John Eccles on the cellular physiology of the cerebellar cortex and was awarded his PhD in 1966. From 1976 he has been Chairman of the Department of Physiology and Neuroscience, Thomas and Suzanne Murphy Professor of Neuroscience, at the New York University School of Medicine, Manhattan, New York. He was elected to the US National Academy of Sciences in 1986.



Left Kazuo Sasaki (1929–) graduated MD in 1954 at the Faculty of Medicine, Kyoto University, and gained his PhD in 1959 from the Graduate School of Medicine of that University. Following appointments in the Department of Physiology in Kyoto, he was a Research Fellow in the Department of Physiology, JCSMR, from 1963 until 1966, carrying out research with John Eccles on the cellular physiology of the cerebellar cortex. From 1970–93 he held a Professorship at Kyoto University, within the Institute for Brain Research (1970–90) and the Graduate School of Medicine (1990–93). In 1993 he joined the National Institute of Physiological Science, in Okasaki, and has been Director-General since 1997. In 1998 he was awarded a Prize of the Japan Academy.

Right Piergiorgio Strata (1935–) graduated MD at the University of Pisa in 1960, and commenced his research career in the University's Department of Physiology with the distinguished neurophysiologist Professor G. Moruzzi. For a year from August 1965 he was a Visiting Fellow with John Eccles in Canberra, and then for a year in Chicago, investigating excitatory and inhibitory interneurons and their connections in the cerebellar cortex. In 1967 he was appointed Associate Professor of Physiology in Pisa, and, in 1975 Professor of Physiology. Later he was appointed Professor of Neurophysiology in the Medical Faculty of the University of Turin, and is currently a member of the Department of Neuroscience, Rita Levi Montalcini Center for Brain Research of the University of Turin. President of the Italian Neuroscience Society (1988–90), Vice-President of the European Neuroscience Society (1988–92), he continues to hold senior positions in IBRO and the Human Frontier Science Program, and has been awarded numerous prizes for his contributions to central nervous system neurophysiology.

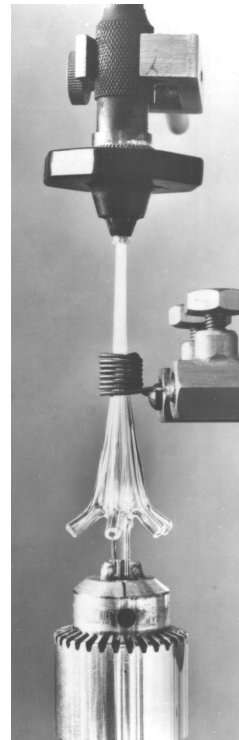
Microelectrophoresis

by David Curtis

Considerable difficulties exist in ascribing the effects on central nervous system neurones *in vivo* of compounds administered intravenously, intra-arterially, topically or by pressure injection, to the activation of specific receptors at synapses on the neurones under observation. Hence techniques were developed from 1957 by David Curtis (see p. 75) to combine the recording of extra- or intra-cellular potentials from single anatomically and physiologically identified central neurones whilst administering compounds of pharmacological interest directly

into their immediate vicinity. This technique was developed as a modification of that previously used by others to administer acetylcholine and other agents close to neuromuscular junctions of isolated muscle preparations. Rather than using pressure to eject pharmacologically active compounds from solutions within fine glass micropipettes, electrical currents were used to control both the leakage and the ejection of active cations or anions from aqueous solutions of appropriate salts.

Skilled glass blowers in the School



Left Teun Van Arkel, photographed in 1981, fusing six glass tubes around a central tube to produce a seven barrel glass microelectrode blank.

Right A seven barrel glass microelectrode blank held in the heating coil of an electrode puller by two chucks, the upper fixed and the lower attached by a vertical rod to the plunger of a solenoid. The melting glass is initially drawn out slowly over a pre-set distance by gravity, and then more rapidly when the solenoid is turned on.

Workshop, including G. Allen, L. Vandeneer, T. Van Arkel and L. Wells, developed techniques to fuse as many as six glass tubes together around a central tube. The resultant seven-barrel micropipette blank was then drawn out using a vertical microelectrode puller designed and constructed in the School. The glass, melted in an electrically heated coil was initially pulled slowly by gravity, and then more rapidly by a solenoid to produce a long tapered shaft with a tip less than one micrometre in overall diameter. After the tip had been ground back to an external diameter of 6-8 micrometres, the individual barrels (orifice diameters 1-2 micrometres) were filled from the top with appropriate solutions, and the assembly then centrifuged to drive the solutions to the orifices of every barrel. Since the central barrel was used as an extracellular recording microelectrode, the assemblies were called multibarrel microelectrodes, and the technique microelectrophoresis (or microiontophoresis).

The central barrel could also be used as a stimulating microelectrode, using brief current pulses of the order of 1-2 microamps to measure the excitability of central nerve fibres, synaptic terminations and neurones in the immediate vicinity of the electrode tip. For intracellular recording a separate single or double barrel glass recording microelectrode was cemented along the side and parallel to the multibarrel assembly, and projecting beyond its tip by 40-100 micrometres in order to impale a single neurone.

Standard anatomical and neurophysiological procedures were used to locate and identify particular types of central neurone, and to record and process extracellular and intracellular potentials. The major significance of the introduction of seven-barrel microelectrodes in Canberra was the opportunity to examine the actions of as many as six different agents on a single neurone. It thus became possible to mimic, antagonize or enhance the actions of synaptically released transmitters. Useful information was provided for identifying the transmitter released by known excitatory or inhibitory

pathways, for determining the nature of the neuronal postsynaptic receptors involved, the changes induced in the postsynaptic membrane, the processes by which released transmitter was inactivated and the mechanism of transmitter release.

This new method of investigating the pharmacology of central synapses *in vivo* was used initially by Curtis and Rosamond Eccles in 1957 to confirm the cholinergic nature of the excitation of Renshaw cells by impulses in motor axon collateral fibres (see p. 414). Subsequently, it was used in the investigation of other potential central neurotransmitters, including amino acids, (see p. 427), and was rapidly taken up by many laboratories abroad.

The major limitation of this technique is the inability to determine the extracellular concentrations of administered compounds which are achieved in nervous tissue. Furthermore, since administration occurs from virtually a point source, concentrations are highest close to the pipette orifice and lower further away, depending on distance, diffusion in the complex extracellular space and the processes which inactivate a particular substance. Thus, when extracellular action potentials are being recorded by an electrode close to the soma of a neurone, the concentration of an administered compound may be too low to influence receptors on distal dendrites. This problem may be overcome by using two multibarrel micropipettes, either firmly attached with a preselected tip spacing or separately manipulated (see p. 434). The latter procedure has also been used to examine the synaptic connections between neurones, one micropipette being used to record from and excite one type of cell and the other to detect the subsequent excitation or inhibition of another type. In studies of this nature, the use of an excitatory amino acid to excite neurones provided a considerable advantage over electrical excitation, since excitatory amino acids, unlike electrical pulses, do not excite axons passing through the region of interest.

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Identification of Central Amino Acid Neurotransmitters

by David Curtis

Elucidation of the nature of the transmitter released at identified central excitatory and inhibitory synapses is essential for a full understanding of the transmission process. Additionally, such knowledge enables the rational development of therapeutic agents with specific effects at particular synapses for treating neurological disorders associated with defective transmission. Knowledge of transmitters is also of value in accounting for the mode of action of known drugs and toxic agents influencing the central nervous system.

A critical observation influencing this investigation was the earlier finding by John Eccles and his colleagues that the convulsant alkaloid, strychnine, blocked the postsynaptic action of the inhibitory transmitter producing 'direct' and recurrent inhibition of spinal motoneurons, inhibitions associated with membrane hyperpolarization (see p. 412). Other short latency and duration spinal postsynaptic inhibitions studied later by David Curtis were also reduced by strychnine.

The appointment in 1958 of Jeffrey Watkins, an organic chemist, initiated a prolonged collaborative investigation which eventually resulted in general acceptance that simple amino acids were the major excitatory and inhibitory transmitters in the mammalian central nervous system. Microelectrophoretic techniques (see p. 425) were used to determine the effects of compounds

known to be present in brain and spinal tissue on physiologically identified spinal, and later other central, neurones. This technique had demonstrated that acetylcholine, widely regarded at the time as possibly an important central transmitter, did not influence the excitability of spinal neurones other than Renshaw cells (see p. 415).

Reports of both the inhibitory effect in crustacea of gamma-aminobutyric acid (GABA), and the unique presence of this amino acid in mammalian brain tissue, led Curtis, John Phillis and Watkins to examine the effects of GABA and related amino acids on neurones in the cat spinal cord. GABA (and glycine) depressed neurone excitability, but hyperpolarization was not detected and the depressant action of GABA was not reduced by intravenous strychnine. Picrotoxin, a GABA antagonist in crustacea, is not ionized in solution and could not be shown in microelectrophoretic experiments to reduce the depressant action of GABA on neurones. Later, the reduction of spinal 'presynaptic' inhibition in the cat by intravenous picrotoxin suggested a role for GABA in this type of strychnine-insensitive inhibition (see p. 417).

In marked contrast to GABA, the acidic amino acids aspartic and glutamic, also present in central nervous tissue, depolarized and excited neurones. These observations resulted in an intensive investigation of the effects on spinal and other central

neurones of numerous structurally related basic and acidic amino acids, some of which were synthesised by Watkins, and in proposals about the possible nature of central amino acid receptors. No evidence was obtained, however, that these naturally occurring depressant and excitatory amino acids were inactivated by extracellular enzymic degradation, as had been demonstrated for acetylcholine. In view of this, and the failure to detect a hyperpolarizing action of GABA, amino acids were considered by the Canberra group in the early 1960s as unlikely to be central transmitters. A particularly significant finding, however, when the structure/activity relationships of excitant amino acids were further explored, was the very potent activity of N-methyl-D-aspartic acid (NMDA), synthesised by Watkins, and later to be of considerable importance in the identification of subtypes of excitatory amino acid receptor.

Late in 1966, Curtis, Graham Johnston, also an organic chemist who was appointed in 1965 to the position previously occupied by Watkins, and colleagues began a reinvestigation of the role of amino acids as central transmitters. This followed the proposal by an American group that glycine was a spinal inhibitory transmitter, based on the association of this amino acid with spinal interneurons and the observation that glycine hyperpolarized motoneurons. In Canberra, an improved technique was developed to combine intracellular recording with extracellular microelectrophoretic administration of amino acids. Both glycine and GABA were shown to hyperpolarize spinal motoneurons with an increase in membrane conductance similar to that produced by spinal inhibitory transmitters. Furthermore, in contrast to GABA, the inhibitory action of glycine was selectively and reversibly antagonized by microelectrophoretic strychnine. On the basis of this antagonism, depressant amino acids were classified as either glycine-like or GABA-like, and, of the former, the neurochemical evidence strongly favoured glycine as the transmitter at strychnine-sensitive inhibitory synapses of spinal 'glycinergic' interneurons. This convincing

evidence for an inhibitory transmitter function of glycine, together with an increasing acceptance that carrier-mediated transport into neurones and glial cells was primarily responsible for maintaining low extracellular levels of amino acids rather than enzymic inactivation, further stimulated a study of central excitatory and inhibitory amino acid receptors.

This was based on the use of compounds having specified stereochemical, conformational and electronic parameters, which selectively mimicked, antagonized or enhanced synaptic transmission. Information was thus obtained about the optimal requirements for interaction with receptors and inactivation processes at synapses studied *in vivo*, and about membrane binding sites, transport systems and metabolizing enzymes studied *in vitro*, for particular amino acids. Since both GABA and glutamic acid are flexible molecules, the finding in 1968 that the heterocyclic isoxazoles muscimol and ibotenic acid had potent and prolonged depressant and excitant effects respectively led to the design, synthesis and evaluation of the actions of numerous conformationally restricted analogues of GABA and glutamic acid.

From 1968 until his resignation in 1980, Johnston and his colleagues synthesised many new compounds for these *in vivo* and *in vitro* investigations. Collaboration from 1974 until 1992 with Povl Krosgaard-Larsen and his colleagues at the Royal Danish School of Pharmacy in Copenhagen also generated many novel and pharmacologically significant compounds. New agents were developed having selective effects on subtypes of receptors and transporters, many of which became available commercially. The discovery by Krosgaard-Larsen and Johnston of nipecotic acid as an inhibitor of GABA transport resulted in the development in Europe of a new class of anticonvulsant drug.

A major breakthrough regarding the possible structure of GABA receptor antagonists followed Johnston's analysis of the structural features of strychnine and other glycine antagonists considered to be essen-

tial for interaction with glycine receptors at inhibitory synapses. As a consequence, in 1970, a number of tetrahydroisoquinolines were tested, of which bicuculline was the most potent convulsant. When administered electrophoretically, bicuculline reversibly antagonized the depression of spinal interneurone firing by GABA-like amino acids, including muscimol, with little or no reduction of the actions of glycine-like amino acids. The first report of this selective antagonism was published in *Nature* on June 27 1970, having been submitted on May 19 by Johnston, Curtis, Arthur Duggan and Dominik Felix. By the end of that year, bicuculline had been shown to block strychnine-insensitive synaptic inhibitions in the thalamus, the lateral vestibular nucleus, the hippocampal, cerebral and cerebellar cortices, the olfactory bulb and 'presynaptic' inhibition in the spinal cord. In combination with neurochemical studies of the presence of GABA and its synthesising enzyme, glutamic acid decarboxylase, bicuculline thus became a valuable pharmacological tool with which to identify central inhibitory interneurons which released GABA as a transmitter.

In 1972 Johnston prepared the more soluble and stable bicuculline methochloride, still widely used as an antagonist of GABA receptors now defined as the GABA-A subtype. Many other convulsants were studied in Canberra and some, including benzylpenicillin, were selective GABA-A antagonists. Others were glycine antagonists. Later, two subtypes of bicuculline-insensitive GABA receptor were defined, GABA-B, activated by (-)-baclofen [(-)-beta-(4-chlorophenyl) GABA], for which specific antagonists and agonists were subsequently developed elsewhere (see below and p. 418), and GABA-C activated by cis-4-aminocrotonic acid, a conformationally restricted analogue of GABA synthesised by Johnston and his colleagues in 1974.

Numerous GABA analogues and other compounds were tested *in vitro* as inhibitors of high and low affinity GABA transport processes. Although some of the most effective, including (-)-nipecotic acid,

enhanced and prolonged the inhibition of neuronal firing by microelectroretic GABA *in vivo*, a direct effect on GABA-mediated synaptic inhibition could not be demonstrated. Anaesthetic barbiturates, however, which increase and prolong synaptic inhibitions mediated by GABA, were shown by David Lodge and Curtis in 1977 to increase and prolong the inhibitory effect of GABA but not that of glycine on spinal neurones. This effect, unrelated to inhibition of GABA transport, was later established to be a direct action of barbiturates and other anaesthetics in the vicinity of GABA-A receptors.

In 1954, Eccles, Curtis and Vernon Brooks had found that the convulsant neurotoxin of *Clostridium tetani* blocked short latency and duration strychnine-sensitive inhibitions of motoneurons. Later, in 1971, Curtis, Felix, Christopher Game and Roy McCulloch demonstrated that tetanus toxin also blocked the longer latency and duration bicuculline-sensitive 'presynaptic' inhibition of spinal reflexes and the accompanying PAD (see p. 416). Furthermore, this toxin also blocked GABA-mediated basket cell inhibition of Purkinje cells in the cerebellar cortex. In contrast, however, to the selective antagonism by strychnine and bicuculline at postsynaptic glycine and GABA receptors respectively, tetanus toxin did not significantly reduce the inhibitory actions of these amino acids on spinal and Purkinje cells. Accordingly, the convulsant action of tetanus toxin was proposed to result from a presynaptic reduction of the release of glycine and GABA at inhibitory synapses.

Recognition of the transmitter function of glycine and GABA, and of the importance of cellular uptake in removing amino acids from the extracellular medium, also led to renewed interest in Canberra in aspartic and glutamic acids as central excitatory transmitters. This was accompanied and made possible by the synthesis of many new compounds by Johnston, and later by Krogsgaard-Larsen, and their colleagues. In 1971, the ionic mechanism of the depolarization of motoneurons by aspartic and

glutamic acids was shown to be similar to that of synaptic excitation. Although several of a large number of analogues and other compounds tested at this time reduced the sensitivity of neurones to both amino acids, a search for a selective antagonist for either naturally occurring amino acid was not successful.

Neurochemical evidence provided by Johnston, Duggan and investigators elsewhere was consistent with glutamic acid being the transmitter of dorsal root primary afferent (sensory) fibres and aspartic acid that of intraspinal excitatory interneurons. This proposal was supported by Duggan's subsequent finding that spinal interneurons excited monosynaptically by afferent impulses were significantly more sensitive to L-glutamate than to L-aspartate. In contrast, the reverse was observed for cells excited polysynaptically but not monosynaptically by afferent impulses. Greater differences in sensitivity were observed in 1973–74 using kainic acid, a conformationally restricted analogue of L-glutamic acid, and NMDA, and Johnston proposed that kainic acid and NMDA were selective agonists of 'glutamate-' and 'aspartate-prefering' receptors respectively. By the late 1970s, the existence of multiple excitant amino acid receptors was clearly becoming apparent, receptor classification depending on activation by NMDA, kainic acid, quisqualic acid or alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and eventually on the increasing availability of selective antagonists. AMPA, structurally related to ibotenic acid, had been synthesised by Krogsgaard-Larsen and shown in Canberra in 1980 to be a potent excitant of central neurones.

By this time, many groups of investigators abroad were becoming involved in this complex field of central excitant amino acid receptors, including Watkins, who played a major role. Although there was interest in discovering specific antagonists for determining the nature of excitant amino acid receptors at particular excitatory synapses, there was the added attraction to pharmaceutical companies of the potential thera-

peutic use of antagonists, particularly as anticonvulsants and as 'neuro-protective' agents. Potent excitant amino acids, such as kainic acid, had been shown under experimental conditions to destroy central neurones but not nerve fibres or synaptic terminals *in vivo*. Subsequent *in vitro* and *in vivo* studies led to proposals that excessive release of endogenous glutamic acid, as during cerebral hypoxia or trauma, or the ingestion of excitatory amino acids able to cross blood-brain-barriers, resulted in the destruction of central neurones. Such 'excitotoxicity' may be important as an aetiological factor in some 'neurodegenerative' disorders and in increasing the amount of neuronal damage produced by trauma or a stroke. As expected, however, from the participation of amino acid excitatory transmitters widely throughout the central nervous system, successful clinical use of antagonists has yet to be achieved, psychotomimetic activity being a major side effect.

Following Johnston's appointment to the Chair of Pharmacology in the University of Sydney in 1980, the reduced 'amino acid group' retained an interest in excitant amino acid neuropharmacology, and collaborated with chemists in Australia and abroad, particularly with Krogsgaard-Larsen. The expanding interests internationally in this field, however, led to the decision to concentrate upon the complex role of GABA in spinal 'presynaptic' inhibition (see p. 417). In 1980, on the basis of the similar effects of GABA and the GABA analogue (-)-baclofen on peripheral tissue preparations, Norman Bowery and his colleagues in the United Kingdom had classified GABA receptors as GABA-A, blocked by bicuculline and not activated by baclofen, and GABA-B, insensitive to bicuculline and activated by GABA and baclofen. Earlier, in 1973, bicuculline had been shown in Canberra not to diminish two central effects of baclofen: the reduction of excitability of spinal and other central neurones, and the reduction of the release of excitatory transmitter from the terminals of primary afferent fibres in the spinal cord. No selective

GABA-B receptor antagonists were then available, however, with which to elucidate the functional significance of these receptors.

The demonstration in the late 1970s by Watkins and his colleagues that omega-phosphonic analogues of glutamic acid were selective NMDA antagonists led in 1986 to the synthesis by David Kerr, Rolf Prager and colleagues in Adelaide of the phosphono derivative of baclofen (phaclofen), and later a sulphonic derivative (2-hydroxy-sulphonic acid). Both were weak baclofen antagonists in the peripheral nervous system, and when tested in Canberra reversibly blocked the presynaptic effect of baclofen on transmitter release in the spinal cord. Baclofen, a GABA analogue which penetrates the blood-brain-barrier, had been initially synthesised in 1962 by CIBA in Basle and had been used clinically for many years to alleviate spasticity of spinal origin in humans. The company had retained an interest in developing agonists and antagonists. Hence, reports in the early 1990s by chemists at CIBA-GEIGY of the synthesis of a series of baclofen agonists and antagonists, which were phosphinic acid analogues of GABA, were of considerable interest. Curtis and Gary Lacey confirmed the agonist or antagonist actions of many of this

series at spinal GABA-B receptors, including those on the terminals of primary afferent fibres (see p. 418). Differences were found *in vivo* between spinal and supraspinal receptors which suggested the existence of several subtypes of central GABA-B receptor, the functional significance of which have yet to be established.

Beginning in 1958, investigators in the Departments of Physiology and later Pharmacology introduced and developed microelectrophoretic techniques to study central amino acid neurotransmission at a cellular level in the mammalian central nervous system *in vivo*, and pioneered the use of numerous compounds, many of which were synthesised expressly for a specific purpose. Apart from glycine, GABA, strychnine, bicuculline, bicuculline methochloride, aspartic and glutamic acids, key compounds of invaluable practical neuropharmacological importance include: AMPA, cis- and trans-4-aminocrotonic acid, NMDA, threo-3-hydroxy-L-aspartic acid, baclofen and baclofen antagonists, isoguvacine, D- and L-homo cysteic acid, ibotenic acid, kainic acid, dihydrokainic acid, muscimol, nipecotic acid, quisqualic acid, L-serine-O-sulphate, 3-aminopropanesulphonic acid, piperidine-4-sulphonic acid and 4-aminotetrolic acid.

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Left Jeffrey Clifton Watkins (1929–) graduated BSc(Hons) in 1950 from the University of Western Australia, and gained a PhD in Organic Chemistry at the University of Cambridge in 1954. After postdoctoral research at Cambridge and Yale Universities, he was appointed to a Research Fellowship in the Department of Physiology JCSMR in January 1958 (Fellow 1961). Until 1965 he worked with David Curtis on the identification of excitatory and inhibitory amino acids as central transmitters, synthesising many new compounds, and then moved to the United Kingdom. At the ARC Institute of Animal Physiology he worked on model biological membranes, and from 1968–73 renewed his interests in central neurotransmission at the MRC Neuropsychiatry Unit at Carshalton. From 1973 until his retirement in 1999, he took up a series of appointments in the Departments of Physiology and Pharmacology in the School of Medical Sciences of the University of Bristol (Honorary Professor 1989) where he concentrated his research on establishing and elucidating the central transmitter role of L-glutamic acid, mainly by developing specific ligands for glutamate receptor sub-types. Elected FRS in 1988, he was awarded the Thudichum Medal by the Biochemical Society (UK) in 2000. He continues to maintain close links with excitatory amino acid research in Bristol.

Right Graham Allan Ross Johnston, AM (1939–) graduated BSc(Hons) in 1959 from the University of Sydney, and gained a PhD in Organic Chemistry at the University of Cambridge in 1964. After postdoctoral research at the University of California in Berkeley he was appointed to a Research Fellowship in the Department of Physiology JCSMR in 1965 (Fellow 1968, Senior Fellow 1972). He collaborated with David Curtis on the neuropharmacology and neurochemistry of central amino acid transmitters until 1980 when he was appointed to the Chair of Pharmacology at the University of Sydney. There he established the Adrien Albert Laboratory of Medicinal Chemistry and continued chemical studies on GABA and glutamic acid as neurotransmitters, developing specific ligands for subtypes of amino acid receptors and transporters. Elected to Fellowship of the Australian Academy of Technological Science and Engineering in 1993, he is the recipient of a number of medals, and in 1998 his services to bio-organic chemistry and pharmacology, to scientific organizations and to science policy development was recognized by appointment as a Member in the Order of Australia.



Povl Krogsgaard-Larsen (1941–) graduated from the Royal Danish School of Pharmacy in 1967 and received his PhD in 1970, DSc in 1980. He was appointed an Associate Professor in the School of Pharmacy in 1970, and since 1986 has been Professor of Medicinal Chemistry. His interests in the structure/activity relationships of neurotransmitter amino acid analogues led to his appointment as a Visiting Fellow in the JCSMR Department of Pharmacology in 1974, 1977, 1981 and 1987 and collaboration with Curtis, Johnston and their colleagues in the testing of a very large number of new and novel compounds synthesised in Copenhagen. He is the recipient of numerous prizes and awards, several Honorary Doctorates, is a member of a number of learned Academies including the Danish Academies of Natural and Technical Sciences, and has been a member of the Board of Carlsberg A/G and of the Board of Directors of the Carlsberg Foundation since 1993. He has a particular interest in encouraging the commercialisation of therapeutically effective compounds.

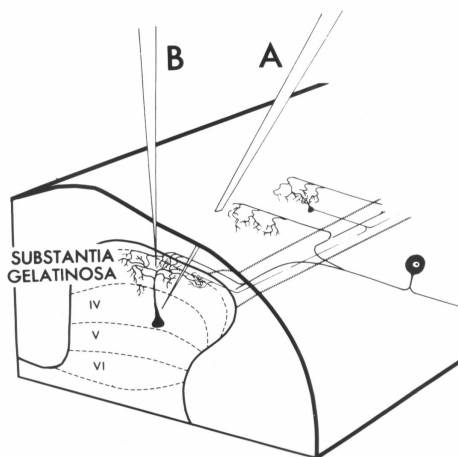
Morphine, Neuropeptides and Pain

by Arthur Duggan

From 1973, Arthur Duggan, together with John Hall, Max Headley, Bernadette Grier-smith and Steve Johnston, investigated the effects of morphine on neurones of central pathways involved in the processing of nociceptive (pain) information with the aim of understanding how opiates relieve pain. They rapidly confirmed previous work that intravenous morphine reduced the excitation of neurones in the dorsal horn of the lumbar spinal cord of anaesthetised animals by peripheral stimuli considered to be painful, but the results of administering morphine microelectroretically near the same neurones were puzzling. Under these latter conditions morphine was a weak depressant of neuronal firing, but this effect

was not mediated by opiate receptors since, unlike the analgesic effect of intravenous morphine, it was not reversed by the opiate antagonist naloxone.

When multibarrel micropipettes are used to both record neuronal activity and administer pharmacologically active compounds, the tips need to be positioned close to the bodies of the neurones of interest (see p. 426). Hence Duggan and his colleagues decided to explore the possibility that opiate receptors were located on or near the dorsally directed distal dendrites in superficial spinal laminae of neurones with cell bodies located deeper in laminae IV and V. Two independently manipulated multibarrel micropipettes were used; one positioned to



Diagrammatic partial cross section of spinal cord showing the arrangement of micropipettes to record the activity of single neurones in dorsal horn laminae IV and V (A) and to administer compounds microelectropheretically more dorsally in the substantia gelatinosa (B). The terminals of a primary afferent fibre are shown synapsing with the dendrites of the deeper neurone.

record extracellular neuronal action potentials in laminae IV and V and the other with its tip more dorsally in a superficial lamina of the spinal cord, the substantia gelatinosa. The neurones were excited alternately by peripheral cutaneous stimulation, noxious by localized heat and innocuous by mechanical deflection of hairs.

These experiments were immediately successful; morphine administered in the substantia gelatinosa powerfully and selectively reduced or abolished the excitation of deeper cells by the noxious stimuli. This action was reversed by naloxone administered locally into the substantia gelatinosa or intravenously. Furthermore, naloxone administered after morphine often produced hyperexcitability of the neurones, suggesting that the process of dependence on morphine had commenced. The opioid peptide methionine enkephalin had the same selective depressant action as morphine. Much subsequent work showed that the opioid receptors in the substantia gelatinosa are

located on small intrinsic neurones of this lamina, which synapse with the dendrites of neurones in deeper laminae, and on the presynaptic terminations in this area of cutaneous nociceptive afferent fibres.

In the same year (1976) that this work was published in *Nature*, a publication from the laboratory of T.L. Yaksh in the United States reported that the intrathecal administration of morphine around the caudal spinal cord of rats produced analgesia restricted to the hind limbs. Together with the investigation in Canberra, this established a powerful case for the clinical use of epidural and intrathecal morphine in humans. This technique soon proved to be very effective, and continues to be used for a variety of purposes including cancer pain, neuropathic pain and during surgical procedures. Sophisticated catheters and pumps have been developed so that patients retain mobility. Relatively high doses can be used at the spinal level without the more general adverse side effects which accompany the systemic administration of high doses of morphine.

This study of the action of morphine led Duggan to investigate other aspects of the physiology and pharmacology of pain. Although there was good evidence that L-glutamic acid was the transmitter released at the first central synapse of rapidly conducting primary afferent fibres from skin and muscle (see p. 430), there were suggestions that the transmitter of slowly conducting fibres which convey nociceptive information was the neuropeptide, Substance P. At that time, the techniques used for collecting and identifying transmitters released in the central nervous system were relatively crude. A perfusing solution was either applied to the surface or passed through a tube inserted into the brain or spinal cord. An entirely new and sophisticated approach was developed in 1985 by Duggan, Ian Hendry (see p. 441) and Robert Morton, in which the outer surface of fine glass micropipettes was coated with antibodies to a neuropeptide of interest using the methods of enzyme immobilization. When inserted into the central nervous system a proportion

of synaptically released neuropeptide molecules bound at discrete sites on the surface of these antibody microprobes. These sites, subsequently detected as deficits in autoradiographs of the probes following incubation in a solution of a radiolabelled form of the particular peptide, were readily related to specific regions in histological preparations of the tissue under examination. A computerized digitizing procedure was developed to average the autoradiographs of a number of microprobes which had been inserted into similar regions of the spinal cord and subjected to the same peripheral physiological stimuli.

Duggan, together with Zhi-Qi Zhao and William Hutchison, soon showed that severe peripheral noxious stimuli released Substance P in the substantia gelatinosa,

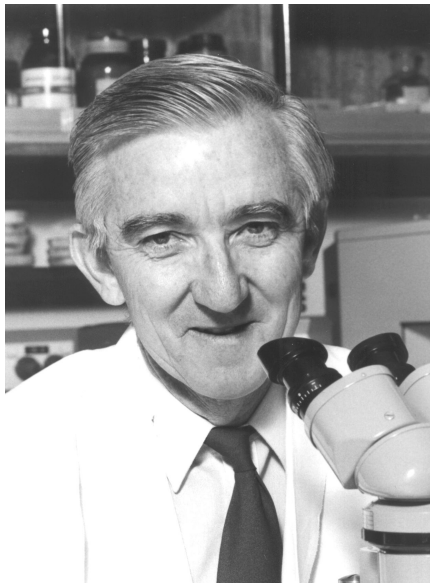
and that it was derived from the terminals of nociceptive afferent fibres. Subsequent investigations demonstrated that inflammation of peripheral tissues caused a massive release of the neuropeptide in the spinal cord, and other investigators implicated this release to hyperexcitability of spinal neurones and the enhanced perception of pain. The available evidence, however, does not favour a role for Substance P as the excitatory transmitter released at the central terminals of nociceptive fibres, but it appears to be co-released with glutamic acid mainly when inflammation occurs peripherally.

The antibody microprobe technique has also been used to study the release of several other neuropeptides in the brain and spinal cord, including beta-endorphin, galanin, dynorphin and neurokinin A.

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Arthur William Duggan (1932–) graduated MBBS from the University of Queensland in 1960, having received a BSc degree in 1958. After seven year's clinical practice, including two years involvement in Brisbane in a study of traffic accident injuries, for which in 1970 he gained a MD degree, he commenced his PhD studies in the JCSMR Department of Physiology in January 1968. His involvement in investigating the spinal effects of inhibitory and excitatory amino acids, of the convulsants strychnine and bicuculline, and of morphine led to the award of his PhD in 1971. From 1971 until 1973 he further developed his interests in amino acid transmitters and opiate analgesics as a C.J. Martin Fellow in the Department of Physiology in the University of Bristol. The third year of this Fellowship was spent in the JCSMR, and in 1974 he was appointed as a Fellow (Senior Fellow 1978) in the Department of Pharmacology, where he continued his investigations of pain pathways, the actions of morphine and of neuropeptides, including endogenous opioid peptides. In 1987 he was appointed Professor of Veterinary Pharmacology in the Royal Dick Veterinary College of the

University of Edinburgh. Ten years later he returned to Australia and is currently Visiting Professor in the Department of Anaesthesia and Pain Management at Royal North Shore Hospital, University of Sydney. In 1994 he was elected a Fellow of the Royal Society of Edinburgh.

Synaptic Transmission in the Mammalian Peripheral Nervous System

by Caryl Hill

Most of the initial investigations of synaptic transmission carried out in the Department of Physiology and subsequently in the Department of Pharmacology were concerned with excitation and inhibition in the mammalian central nervous system. There was, however, an early interest in excitation in autonomic ganglia and at the neuromuscular junction, synapses which previously had been used by numerous neurophysiologists, including John Eccles, as more readily investigated models of transmission mechanisms within the central nervous system.

In 1955, Rosamond Eccles (see p. 415) reported the first successful intracellular recording of resting, action and excitatory synaptic (junction) potentials from neurones in rabbit superior cervical ganglia. William Liley (1929–1983) and John Hubbard (1930–1995), both of whom had been BMedSc students with Eccles in Dunedin, studied excitatory transmission mediated by acetylcholine at neuromuscular junctions in the rat diaphragm. Liley, a PhD Scholar in the JCSMR from 1955 to 1957, examined the spontaneous and evoked release of transmitter by recording and analysing ‘miniature’ and end-plate potentials. Hubbard, a PhD Scholar 1958–1960, Research Fellow 1961, Fellow 1962 and Senior Fellow 1964–1967, alone and with a number of colleagues, investigated the electrophysiological properties of motor nerve terminals, the factors which influenced acetylcholine release by nerve impulses, post-activation potentiation of release and the relation between release and the intracellular distribution of synaptic vesicles.

Following the appointment of David Hirst in 1981, another aspect of autonomic neurotransmission, transmission at the terminals of ganglion neurones on the smooth muscle cells of arteries, arterioles and the gastrointestinal tract, was intensively investigated. Hirst, in collaboration with Dirk

van Helden, examined the properties of smooth muscle cells in arterioles. The resting membrane potential of these cells, hitherto considered to be regulated by the activity of a sodium/potassium pump, was found to be essentially determined by the activity of an ion channel selectively permeable to potassium ions. In collaboration with Alan Finkel of the Experimental Neurology Unit, who had developed a single electrode voltage clamp (see p. 453), direct measurements were made of the time course and properties of the membrane currents underlying excitatory junction potentials produced by stimulation of the perivascular sympathetic nerves. This was a very significant step forward in understanding the transmission process at sympathetic nerve terminals.

Mutual interests in sympathetic innervation and neurotransmission led to a collaborative study by Caryl Hill, Hirst and van Helden of the development of functional synapses in arteries and arterioles. The earliest postnatal vascular responses to sympathetic nerve stimulation were found to differ physiologically and pharmacologically from those recorded later during development. This suggested that these blood vessels could be controlled by the sympathetic nervous system early during development, prior to the formation of mature synapses, and that sympathetic nerves might have a trophic influence in shaping the nature of the mature postsynaptic response. Subsequent investigations included an electrophysiological and anatomical study of the restoration of sympathetic responses during neural regeneration, and a pharmacological and anatomical analysis of the development of the sympathetic innervation of enteric neurones of the gut, the latter in collaboration with Meng-Chong Ngu of the Department of Clinical Science. Together, these studies confirmed the selectivity of the

processes underlying innervation by sympathetic nerve fibres during development and regeneration.

In a series of experiments in collaboration with Gerald Silverberg, visiting from Stanford University, in 1984 Hirst, van Helden and Hill provided evidence that sympathetic innervation could indeed have a trophic influence on the function of vascular smooth muscle. Using cerebral arterioles, experiments were aimed at measuring the kinetics of smooth muscle voltage-dependent calcium currents which are responsible for muscle action potentials and contraction. A direct correlation was found between the density of innervation and the voltage dependent excitability of the smooth muscle cells, suggesting that the density of calcium channels was in some fashion influenced by the pattern of sympathetic innervation.

Hill's interest in autonomic neurotransmission during development, together with her continuing studies on neuronal growth factors in the laboratory of Ian Hendry (see p. 441) led to a comparison of the sympathetic innervation of different target tissues in the gastrointestinal tract. Different subpopulations of sympathetic neurones were found to exist from the earliest stages of neurite outgrowth, which innervated different target tissues and depended on different trophic factors for their survival during development. This finding, in 1985, was one of the earliest demonstrations that sympathetic ganglia comprised a heterogeneous population of neurones projecting axons to appropriate target cells with a great deal of precision. Subsequently, the axonal processes of sympathetic neurones were shown to branch in the vicinity of target cells, the excess collateral branches being gradually pruned over time. Neuronal competition was identified as an important factor in this reduction of collaterals during development.

Following the 1988 JCSMR Review, Hill established an independent laboratory in order to extend her studies of autonomic neurotransmission in a variety of target tissues. Molecular biological studies concern-

ing the expression of transmitter receptors during development were commenced in 1991 with Maria Vidovic. The aim was to determine whether the physiological and pharmacological changes in synaptic responses observed during development were due to neural induction of receptor expression. Comparative data from different tissues showed, however, that receptor expression occurred before there was a significant entry of nerve fibres into target tissues, and was maintained in tissues which had been denervated at birth. Thus, modifications in receptor types mediating functional responses of target cells were postulated to result from selective interaction of the nerves with appropriate receptors on smooth muscle cells. This proposal is consistent with a later demonstration by Shaun Sandow that very close associations between the transmitter release sites and the postsynaptic muscle membrane are present at the times when responses of an immature type can be recorded. Thus, modification of physiological responses during development may result from close range interactions and the possible interchange of growth factors.

Another major investigation during the 1990s concerned the receptor-activated signal transduction mechanisms initiated by perivascular sympathetic nerve stimulation, undertaken by Hill and Dianna Gould. The findings indicated that the contraction of smooth muscle in some small arterioles was the consequence of the release of intracellular calcium rather than, as generally accepted, the influx of calcium through voltage activated channels in the muscle membrane. This was a particularly important observation which suggested that drugs designed to reduce the constriction of small arterioles by blocking these calcium channels may not be very effective, and pointed to the need to develop drugs aimed at the intracellular calcium store/release process.

These results, and those from other laboratories, have provided evidence for the heterogeneous nature of the physiological mechanisms and receptors involved in auto-

onomic nerve-mediated responses in different vascular beds. Hill and Jacqueline Phillips made a comparative study of mRNA expression of receptors mediating vasoconstriction and vasodilation in four different types of blood vessel. Differential gene expression appears to be important in the heterogeneity of vascular responses since a unique array of receptors is present in each type of vessel, some expressed in common, others specific for a particular type of vessel.

Another factor determining the heterogeneity of vascular responses is structural diversity within the vascular wall. Different sized vessels have different numbers of lay-

ers of smooth muscle cells, and the coordination of vasoconstriction and vasodilation relies on cellular coupling through structures called gap junctions. These are ion channels comprised of constituent molecules called connexins. Recent evidence indicates differences between the incidence of gap junctions and the subtypes of the constituent connexins in different vascular beds and different sized vessels. Genetically modified mice are to be used to further examine the role of gap junctions and connexins in determining vascular responses controlled by the autonomic nervous system in health and disease.

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George David Scarcliffe Hirst (1942–) graduated BSc Pharmacy in 1965, BSc Pharmacology in 1966 and PhD in 1969 at the University of Leeds. After a two year postdoctoral fellowship at the University of Edinburgh he moved to Monash University in 1971 and held a series of appointments, including a Queen Elizabeth II Research Fellowship (1973–75) and a Senior Lectureship (1978–81). From 1981–1984 he was a Fellow in the Department of Pharmacology JCSMR, and in 1985 returned to Melbourne, initially as a Visiting Scientist at the Baker Medical Research Institute. Subsequently, he was appointed a NHMRC Senior Research Fellow (1985–1988), Principal Research Fellow (1989–1992) and Senior Principal Research Fellow (1993–) in the Department of Zoology, University of Melbourne. His seminal contributions to autonomic neurotransmission led to his election to Fellowship of the Australian Academy of Science in 1993.



Caryl Elizabeth Hill (1949–) graduated BSc(Hons) from the University of Melbourne and gained her PhD in Zoology in 1974. After a postdoctoral appointment in the Research School of Biological Sciences, ANU, and an Overseas Research Fellowship of the National Heart Foundation of Australia at University College London, she joined Ian Hendry's group in the Department of Pharmacology, JCSMR, in 1978 as a NHF Research Fellow to study neuronal growth factors. Appointed a Research Fellow in 1982, Senior Research Fellow in 1985 and Senior Fellow in 1994, she has been Leader of the Autonomic Synapse Group in the Division of Neuroscience since 1995.

Nerve Growth Factors

by Ian Hendry

In 1974 Ian Hendry introduced a new line of research in the Department of Pharmacology, the study of the factors which determine that developing neurones make contact with the correct target cells. Nerve growth factor (NGF) was the first protein shown to promote the growth of developing neurones, and Hendry had demonstrated in Cambridge that the retrograde axonal transport of NGF was critical in the development *in vivo* of mature adrenergic neurones of sympathetic ganglia. At the time, NGF was generally considered to act as a mitogen, a proposition which was challenged by the findings that this protein enhanced the survival and hypertrophy of neurones, but not their hyperplasia, and did not increase the cellular mitotic index. Subsequently, in

Canberra, Hendry and Sue Ebbott showed that NGF was also retrogradely transported in axons of the central nervous system. As further information became available, the general term 'retrophins' was proposed in 1980 to describe neurotrophic factors released by target tissues which, after retrograde transport, were responsible for the survival of the neurones transporting them.

In collaboration from 1975 with Caryl Hill, then a Postdoctoral Fellow in the Research School of Biological Sciences and with previous experience of growing dissociated sympathetic ganglion neurones with their target tissues in culture, Hendry studied the factors controlling the phenotype of developing neurones. Under tissue culture conditions, these normally adrenergic neurones were

found by enzyme assays to be cholinergic. Following the appointment of Hill to the Department in 1978, a factor released by the non-neuronal cells in the culture was shown to be responsible for this change in phenotype. In 1980, glucocorticoids were found to modulate the nature of the neurones in developing sympathetic ganglia by suppressing the formation of this factor.

Nerve growth factors for parasympathetic (cholinergic) neurones had been demonstrated to be present in muscle in 1977 by Ian McLennan. In the following year he showed that removal of limb buds in foetal animals resulted in the death of motoneurones which would have innervated the missing limb. This observation, together with the finding that there was a factor in cardiac tissue that supported the survival of parasympathetic neurones, led to an attempt to purify the nerve growth factor for motoneurones which are also cholinergic neurones. A high molecular weight complex protein was isolated from bovine heart muscle that promoted the survival of parasympathetic neurones in tissue culture, and a monoclonal antibody was generated that blocked this *in vitro* effect and the development of the parasympathetic system in mice. In 1989, this factor was shown to be identical with 'acidic fibroblast growth factor', a protein with diverse effects acting as a mitogen for fibroblasts and vascular endothelium and as a survival factor for neurones.

By this time a number of neurotrophic factors had been described, and Hendry and his colleagues returned to their earlier interest in the role of retrograde transport in neuronal development. In collaboration with Perry Bartlett and Mark Murphy, of the Walter and Eliza Hall Institute in Melbourne, the retrograde transport of 'leukaemia inhibitory factor' by the sensory nervous system was demonstrated in 1992.

Further progress followed the appointment in 1988 of Michael Crouch (see p. 267), who brought expertise in second messenger systems involved in the actions of growth factors. GTP-binding proteins were shown to be involved in the survival of neu-

rones induced by neurotrophic factors. This, coupled with the finding that some of these factors were not transported retrogradely, led to the concept that retrogradely transported long-acting second messengers convey information from target tissues to innervating neurones. From 1992 Crouch concentrated on growth factors which regulated cell proliferation. These studies suggested that G-proteins might function as intracellular signalling molecules. Subsequently retrograde axonal transport of the alpha sub-unit of GTP-binding proteins was demonstrated, and in 1994 GTP-binding proteins were shown to be translocated to the nuclei of sensory neurones after retrograde axonal transport.

In order to explore the role of the GTP-binding protein Gz, Hendry and his group, in collaboration with Klaus Matthaei (see p. 464), used molecular biological techniques to generate a line of mice deficient in the protein Gz. Although no obvious abnormality of the nervous system could be detected, this animal was hypertolerant to opiates and the project is continuing.

From 1994 a detailed analysis of the second messengers which were transported retrogradely was undertaken, a project which included Sven Johanson, Selina Bartlett and Anna Reynolds. A number of second messengers were found to be involved, not only by being transported themselves like GTP-binding proteins but also in the regulation of the actual transport process. Use was made of the retrograde transport of iodinated NGF from the anterior chamber of the eye to the sympathetic and sensory ganglia to assess the effect of many compounds which modify the second messenger cascades. The importance was shown of the enzyme phosphatidylinositol 3-kinase for neuronal survival and retrograde axonal transport. In later experiments by Shaun Sandow using gold-labelled NGF to examine the ultrastructure of the organelles containing NGF, and by Michael Weible and Kathrina Heydon using rhodamine-labelled NGF to examine the *in vivo* movement of transported particles, a description of a retrograde signalling organelle at the electron

microscopic level became possible. The elucidation of the proteins contained in this structure and its formation at the nerve ter-

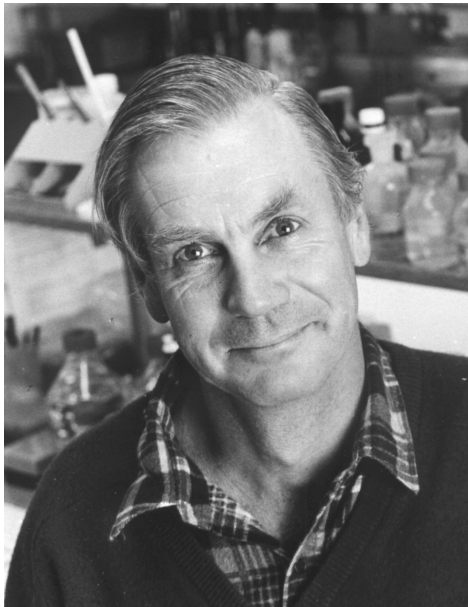
minal will be essential to the understanding of the role of retrograde axonal transport in neuronal survival and differentiation.

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Ian Alexander Hendry (1944–) graduated BSc(Med) (1965), MBBS (1969) and DSc (1992) from the University of Sydney. After a year's junior residency he sought a career in research, obtaining a PhD from the University of Cambridge in 1972. After postdoctoral fellowships at the Biozentrum of the University of Basel and Stanford Medical School, California, he returned to Australia in 1974 as a Queen Elizabeth II Fellow in the JCSMR Department of Pharmacology. He was appointed a Senior Research Fellow in 1976, Senior Fellow (1981), and Leader of the Developmental Neurobiology Research Group in the Division of Neuroscience (1989). In 1995 he was appointed Professor in this Division.

The Neurophysiology of Vision

by Peter Bishop

From the time Professor Peter Bishop (see p. 82) arrived at the John Curtin School in 1967 the neurophysiology of vision became a major component of the research of the Department of Physiology, with concerns about visual optics, the formation of images

in the eye and the organization of the visual system as a whole.

The concept of the receptive field and its properties formed the basis of much of this work. The receptive field of a single neuron is defined as the region in space where

objects cause an increase or decrease in the rate of a cell's discharge. Through their receptive fields, striate cells in the cerebral cortex respond to particular features of objects, such as lines, edges, corners and so on. It is only much later along the visual pathway in the brain that cells respond to complete objects such as the human face.

Based on the concept of receptive field disparity, he, with his colleagues at Sydney University, had already established the basic neural processes underlying binocular single vision and depth discrimination (stereopsis), and at the John Curtin School in 1967 he made binocular vision a major component of the Department's research endeavour. This short essay gives a brief outline of this aspect of the research. Until 1975 most of his research on this and other aspects of vision was done in collaboration with Geoff Henry (see p. 448) and other colleagues, but after that Henry worked independently.

Because the two eyes are horizontally separated in the head, each eye sees a given object from a slightly different vantage point, leading to small horizontal differences in the relative positions of their images on the retinas of the two eyes. Hence the concept of receptive field disparity. The impulses from retinal ganglion cells with receptive fields in the two eyes travel along the optic nerves and pathways in the brain before finally coming together in single cells in the primary visual cortex - the striate cortex. In this way each binocularly activated striate cell responds selectively to one and the same object feature in each eye (binocular single vision) even though the location of the images on the two retinas may not correspond exactly. Each striate cell necessarily has the same response properties from the two receptive fields, although there are cell-to-cell differ-

ences that depend upon the object being viewed.

When the two eyes are turned towards a point in space the images of that point occupy the same relative position on the two retinas, i.e., they are corresponding retinal points. A frontal plane positioned to pass through this fixation point then defines the projection of other corresponding pairs into space and may be used as a reference plane. Points lying closer or further than this plane fall on non-corresponding or disparate parts of each retina.

In the population of striate neurons some fire with a maximum response to objects on the reference plane while others show a preference for closer or more distant points. Thus each striate neuron responds selectively to an object feature located at a particular depth. Work in the JCSMR laboratories showed that for each cell the maximum response occurs when the receptive fields for each eye was in spatial register. Also that the cell's response dipped dramatically with small changes from this in-register position. This narrow range of effective responses brings precision to cell's capacity to estimate depth, and our work did much to explain how cortical inhibitory mechanisms act to restrict the out-of-register responses. With the in-register location varying from cell to cell, the population of striate neurons provides for a precise estimate of a wide range of different depths.

All the above observations were made on the anaesthetised cat preparation and it is clearly beyond the scope of this brief essay to give the experimental details on which the observations were based. One detail that reveals the precision of the system, however, is that there is a significant change in the discharge rate from striate cells when its two receptive fields are out of register by as little as 5 min. arc.

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Highlights of Retinal Research

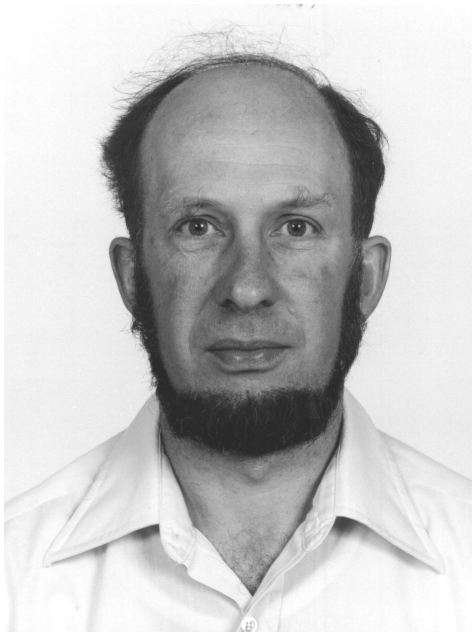
By William Levick

The initial processing of visual information occurs in the retina, a complex laminated structure of rod and cone photoreceptors, interneurons and retinal ganglion cells (RGCs). The axons of ganglion cells, via the optic nerve, the optic decussation and the optic tract, synapse with neurons in the lateral geniculate nucleus (LGN), the axons of which project via the optic radiation to regions of the cerebral cortex concerned with vision. Within the Department of Physiology from 1967–1996 William Levick (see p. 133), and from 1970–1976 Jonathon Stone, investigated RGCs and their central linkages

Specificity of Retino-Geniculate Connections

The very essence of organization in the central nervous system resides in the patterns of interconnections made by individual neurons at different functional levels. Since

the late 19th century such patterns have been inferred indirectly, and often incorrectly, from morphological studies. For example, in the LGN, the main link between retina and visual cortex, it was known that individual LGN neurons were studded with thousands of synapses. This had led to the belief that the LGN was a major centre of integrative activity. An entirely different picture emerged from a neurophysiological attack on the issue carried out by William Levick, Brian Cleland and Mark Dubin in 1971. By making simultaneous single-cell recordings from an LGN neurone and a succession of RGCs, it was established that essentially every output impulse from the former was attributable to an incoming impulse from one (8% of dual recordings) or just a very small number (up to 5) of RGCs. In one stroke, the notion of massive convergence was swept aside. A large proportion of the thousands of synapses on an



Brian Geoffrey Cleland (1937–) received his BE degree from the University of New South Wales in 1959, and MS (1964) and PhD (1967) from Northwestern University, Illinois, where he carried out research on fundamental visual properties of retinal ganglion cells with R.W. Jones and Christina Enroth-Cugell. After a postdoctoral fellowship at Northwestern University for a further year, he was appointed to the Department of Physiology, JCSMR, in 1968 as a Research Fellow, and was promoted Fellow in 1972 and Senior Fellow in 1981. His major research interests included retino-geniculate connectivity, interneurons of the lateral geniculate nucleus and the gradients of visual acuity of retinal ganglion cells. In 1983 he moved to the Department of Physiology at the University of Sydney as a Senior Lecturer. In 1990, after a secondment into the electronics industry, he took up a permanent position with Telectronics Pty Ltd (Sydney).

LGN neurone must be coming from only a single RGC or a very small number of them.

This result attracted wide and persistent attention because it provided much-needed linkages between methodologically different fields. The linkage with morphology came through the identification of sustained and transient receptive field classes with the classical axonal conduction velocity groups (T1, T2) in the primary optic pathway and thus with axonal diameter and cell body

size. A linkage with visual psychophysics came from the identification with the linear/nonlinear (X/Y) classification of ganglion cells and thus with the popular notion that the visual system operated to some extent as a Fourier analyzer (necessarily linear) of the visual scene. A linkage with cortical neurophysiology came from the demonstration that the sustained and transient classes were separately projected to primary visual cortex.

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Functional Classification of Retinal Ganglion Cells

The notion of 'receptive field', originating in the 1930s, is a fundamental concept in the neurophysiological account of the mechanism of so-called 'early' vision. In its simplest form, the concept is expressed as 'the patch of visual field within which the impulse discharge of a visual neurone may be influenced by presentation of a visual stimulus'. At the retinal level, much more is nowadays implied by the concept, largely as a result of the unfolding, in the laboratories of William Levick and Jonathon Stone, of the wide diversity of RGC classes. Thus the current textbook bundling of ganglion cells into just two classes (On-centre and Off-centre) dating from the 1950s is long overdue for revision. Depending on how they are counted, there are upwards of thirteen different classes of cat RGCs.

There are several key factors that under-

pinned the new view. Perhaps the most important was the evaluation of a sufficiently large reference sample of recordings (thousands of contact hours) so that rarely encountered classes could be convincingly distinguished from artefactually damaged versions of commonly encountered classes. Another key realization was that recording 'encounters' cannot be equated with anatomical prevalence. The anatomically numerous (~45%) small-bodied cells present much smaller cross-sectional areas to fine recording electrodes of all types. Yet the dendritic trees of the individual members of each class may be sufficiently large as to completely tile the retina and so supply a complete veridical image-analysis message to higher centres. This is an important feature of a genuine class. What the multiple classes are doing is supplying a multidimensional analysis of the visual scene to higher centres via an inherently parallel visual pathway.

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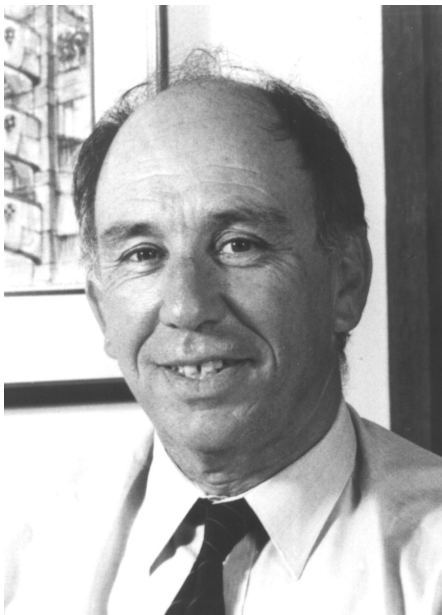
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Optic Decussation

Decussations in the central nervous system are enigmatic morphological arrangements in which collections of similarly sourced nerve fibres from one side meet their mirrored counterparts from the other side at the midline as they cross to the opposite side of the brain or spinal cord. The decussation at the optic chiasm of mammals has a remarkable specialization in that it is incomplete in species with more frontally directed eyes. This feature was recognized more than 270 years ago by Isaac Newton as being associated with the possibility of binocular single vision. He noted that points in the temporal 'half-retina' of the right eye shared similarly directed lines of sight in the external visual world as corresponding points in the nasal 'half-retina' of the left eye. It would therefore make sense for nerve fibres of temporal origin to remain uncrossed at the decussation so as to keep company with the crossing nasally originated fibres of the opposite eye having similar visual directions.

The neurophysiological demonstration of the coming-together of signals from

crossed and uncrossed pathways on single neurones of the visual cortex came 230 years later. It constituted the basis for the most evident aspect of binocular vision, namely, although two eyes are doing the viewing, the percept we experience is of a single visual world. A second, more subtle aspect of binocular vision is that the percept has a three-dimensional character (stereopsis). The neurophysiological basis for this was developed in Peter Bishop's laboratory (see p 442) and elsewhere. The recognition of the many classes of retinal ganglion cells led to the exploration of their individual decussation patterns in the laboratories of William Levick and Jonathon Stone. Surprisingly, it emerged that at least three different patterns existed. This result led to the proposal that the role of one of the classes, 'Brisk-Transient(Y)', in binocular vision is to provide the basis for single vision and stereopsis in a region closer to the subject than that provided by the more numerous 'Brisk-Sustained (X)' class. Ten years later, this proposal received resounding independent support from a study at the LGN and cortical levels.



Jonathan Stone (1942–) graduated BSc Med (1962), PhD (1966) and DSc (1977) from the University of Sydney and began his research career in visual neurophysiology with Peter Bishop in Sydney University's Department of Physiology. A Lectureship in the Department was followed by three postdoctoral years abroad, and in 1970 he returned to Australia as a Research Fellow in the Department of Physiology at JCSMR (Senior Research Fellow, 1972–76). Here he continued his interests in the functional properties of subclasses of retinal ganglion cells, and of neurones in the lateral geniculate nucleus and visual cortex, which led to the development of the concept of parallel processing in the visual system. From 1976 he was successively Senior Lecturer, Associate Professor and Professor in the School of Anatomy, University of New South Wales and in 1987 was appointed Challis Professor in the School of Anatomy (Head of Department, 1987–92) in the University of Sydney. Since 2000 he has been Head of the Department of Anatomy and Histology, and from 2001 Director of the Institute for Biomedical Research in the University of Sydney. His many contributions to vision research were recognized in 1984 by his election as a Fellow of the Australian Academy of Science, of which he was Biological Secretary from 1986–1990.

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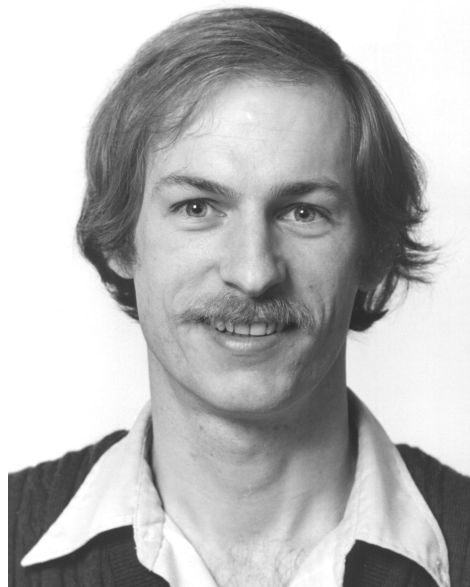
Neurophysiology of a Scotoma

Clinically, a scotoma is defined as a blind patch in the visual field. Pathological scotomata may be associated with a disease process at any level of the visual system from retina to visual cortex. The surprising feature of a scotoma is that it is often hardly noticeable to the subject, even when as much as half the visual field is blanked out ('hemianopia'). Accounting for the percept of a scotoma in terms of neurophysiological mechanisms at the level of the visual cortex or lateral geniculate nucleus is subject to considerable uncertainties because of lack of knowledge of what is happening at the retinal level. The condition of feline central degeneration presented William Levick and Larry Thibos with an exceptional opportunity for the required analysis. In its mature stabilized state the lesion is characterized by

an essentially complete loss of photoreceptors over a sharp-edged patch of central retina. The brightly reflecting tapetum enabled clear visualization of the lesion outline by ophthalmoscopy and back-projection into the external visual field. Moreover, it has long been known that the receptive fields of ganglion cells are approximately centred on the cell-body recording. Thus, in contrast to studies at higher levels, directly visualized placement of the recording electrode enabled precise selection of cells for study with dendritic trees (and expected receptive field centres) in known relation to the edge of the lesion. Measurement of antidromic latency enabled inference of receptive field class, even when visual performance was corrupted.

The main result of the work was that ganglion cells with cell bodies more than 1° inside the border of large lesions were blind,

Larry N. Thibos (1947–) received his BS (1970) and MS (1972) degrees in Electrical Engineering from the University of Michigan and PhD (1975) in Physiological Optics from the University of California, Berkeley, where he carried out research on the retinal processing of spatio-temporal intensity variations. After working as a postdoctoral fellow at the National Institutes of Health, Bethesda, in 1975 he joined the Department of Physiology, JCSMR, as a Visiting Fellow. He was appointed as a Research Fellow (1977–1982) and to a Sulman Fellowship in 1983. His major research interests included information processing by the retina and the role of retinal organization and visual optics in setting the limits to visual performance. In 1983 he moved to the School of Optometry, University of Indiana as Assistant Professor of Visual Science, with promotions to Associate Professor in 1989 and Professor of Optometry and Visual Science in 1994.



although not always silent. Over an approximately 2° wide transition zone centred on the lesion edge the majority of cells had variably reduced visual sensitivity. Thus, although the edge of the photoreceptor lesion is sharp, the representation of it in the ganglion cell array was fuzzy. That the fuzziness was associated with the size of the receptive field centres was clearly brought out by a simple modelling of the effects of variable truncation of receptive fields by the lesion. A further important result was that ganglion cells with the normal distribution of axonal conduction properties were found in plentiful number and ostensibly normal proportions (judged by conduction groups) within long-standing (years) lesions. This indicates that ganglion cell survival in the absence of photoreceptors is not critically dependent on hypothetical anterogradely

transported trophic factors that may emanate from the photoreceptor layer.

The effects at the cortical level of this particular type of lesion have not been studied. Instead, others have created localized lesions of the photoreceptor array by means of a high-powered laser. A strikingly different picture has emerged in that a sizeable proportion of cortical neurones that would normally receive visual input from the affected region instead become sensitive to a patch of visual field (ectopic receptive field) positioned outside the boundary of the lesion. Whether this cortical adult topographic plasticity is related to the character of the photoreceptor lesion (laser ablation is hyperacute; central retinal degeneration takes months to years) is the subject of further ongoing investigations.

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Discoveries in Visual Neuroscience

by Geoffrey Henry

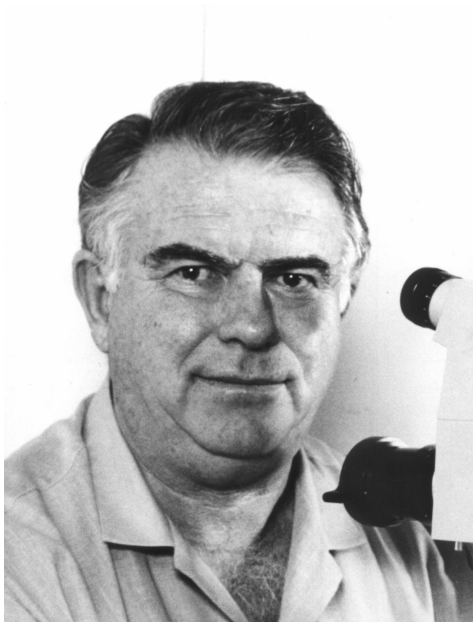
Working with a succession of talented Visiting Fellows, Geoffrey Henry made a number of fundamental discoveries and one important practical discovery in visual neuroscience. Two of the fundamental discovery and the practical discovery are described in the following three essays

The Discovery of New Visual Cells in the Brain

In January 1968 Geoff Henry began to work in association with Peter Bishop to classify the response characteristics of individual

cells in the primary visual area of the cerebral cortex. A year later they were joined by Bogdan Dreher from Poland.

After leaving the eyes, signals coming into the brain undergo their first major processing step in the primary visual area of the cerebral cortex, which covers the surface of the occipital lobe at the back of the brain. To understand how information is processed at this early stage, it is essential to class individual cells from their visual responses. In attempting this task the Canberra group came up against the prevailing classification



Left Geoffrey Herbert Henry (1929–) graduated in science and optometry from Melbourne University in 1952, MAppSc in 1965 and DSc in 1975. During the 15 years from 1952, he practiced optometry, undertook postgraduate studies and was Chairman of the College of Optometry in Melbourne University. Awarded a Churchill Fellowship (1965) to study at Indiana and Cambridge Universities, he began his academic career in 1968 with his appointment to work with Peter Bishop at JCSMR. This association continued until 1976 and in 1979 he was appointed to the position of Senior Fellow. In 1991 he was appointed Director of the Centre for Visual Science at ANU. He retired from JCSMR in 1994. Henry was awarded the Glen Fry Award of the American Optometric Association, the Rimpac Award and made an Honorary Fellow of the National Vision Research Institute of Australia. He is now a Visiting Fellow at ANU.

Right Bogdan Dreher, (1941–) graduated MSc from the University of Warsaw and received his post-graduate training at the Nencki Institute of Experimental Biology in Warsaw (PhD 1968) where he was a Research Fellow for two years before filling a similar position at the JCSMR from 1968 to 1972. He then went to Sydney University in 1972 where he progressed from Research Fellow in the Physiology Department to be made Professor in Visual Neuroscience in 1995. He was awarded a DSc from Sydney University in 1989. His interests have remained in the visual system but have ranged from the embryological development of retinal receptors to the response characteristics of cells in the highest centres of the visual cortex. He is now regarded as a world authority in these fields and is great demand as an international visitor, lecturer and author.

that had been proposed by Hubel and Wiesel, who were to receive the Nobel Prize in 1981. In this classification, each cell's response was interpreted from its perceived position in a functional hierarchy. The acceptance of this scheme virtually closed the door on the discovery of new cell types, since it only allowed for simple cells to provide the input to complex cells which in turn fed onto hypercomplex cells.

The JCSMR group, however, refused to

be hamstrung by this restriction and, during the time of Dreher's stay, they proposed several new cell types and demonstrated that the hierarchical theory did not tell the whole story. The first of the new cell types did not run counter to the hierarchical scheme since three varieties of the simple cell were detected, each displaying distinctive types of binocular interaction. The next new cell, however, was damaging to the scheme since its properties met the

requirements of an implausible cell, one that belonged to a simple hypercomplex class. This provoked hostility from the advocates of the hierarchy but they were placated when, some years later, the authors of the scheme also found these cells.

Another cell type also posed problems since its properties appeared to be those expected from a mix of simple and complex cells. Later experiments in Henry's laboratory, with Alan Harvey and Jenny Lund, showed that these cells projected to a higher cortical area where they terminated on a cell other than one with hypercomplex proper-

ties. It was also demonstrated later by Jean Bullier and Henry that complex cells were frequently the first cells in the processing sequence in the primary visual area.

These results provided evidence that if the hierarchical scheme was to prevail, then other flow paths must exist to bypass different stages of the sequence. The role of these abbreviated paths has remained a mystery, but one that will need to be resolved in order to understand the entire contribution of the primary visual cortex in the act of seeing.

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The Paths Taken by Visual Streams Entering the Brain

From the early days of the visual science program at the JCSMR it had been demonstrated that information emerging from the eyes passed into the brain along a number of parallel streams. One question remained unanswered, what happened to these streams once they entered the visual cortex?

Jean Bullier and Henry set out to resolve this question. Their approach was to initiate a signal (a nerve spike) at one point along the incoming stream and then attempt to record the new signal at another point, either further up or down the stream. The time of arrival and the shape of the signal gave clues of the stream-carrying signal and also the number of cells it had traversed in reaching the recording site. Usually the recording electrode was close to the body of a cortical cell and it was also possible to

classify the cell according to its visual responses.

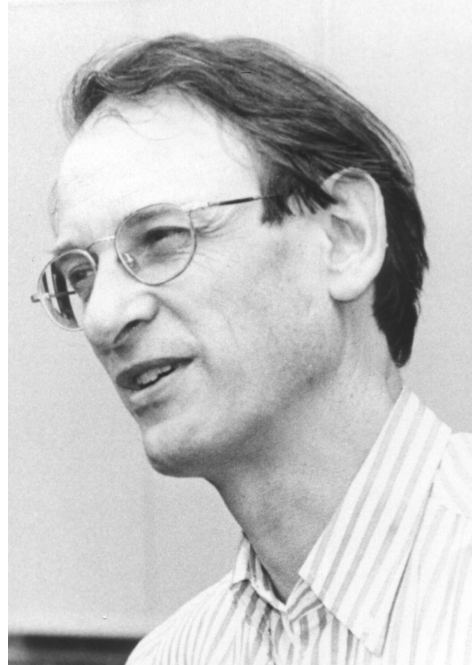
These physiological experiments were complemented by histological experiments applying a similar principle. A tracer substance such as the enzyme, horseradish peroxidase, was injected into a pathway or stream and followed in microscopic sections as it moved away from the injection site. The size of the revealed fibres allowed the stream and its destination to be identified.

These studies opened the way to almost all the modern concepts of flow paths through the visual cortex. Tables were prepared setting out the response characteristics for cortical cells in different streams and at different stages in the processing sequence in the primary visual area. The accuracy and comprehensive nature of these tables has ensured that they have been widely adopted and that they have retained their currency to the present day.

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Jean Bullier, (1949–), a graduate of the École Supérieure d'Electricité in Paris, graduated PhD from Duke University in North Carolina, and then spent three years from 1978 in Geoff Henry's laboratories in the JCSMR. He examined the flow pathways of the different visual streams as they entered and distributed throughout the primary visual area of the cerebral cortex. Eventually this became a lifelong study for Bullier, although he has not stopped in the primary visual area but has carried his interest on into higher visual centres in the brain. Between 1981 and 1998 he worked in the INSERM laboratories in Lyon. In 1993 he rose to be Directeur de Recherche and head of the team working on 'Structure et fonction du cortex visuel'. He was awarded the Silver Medal of the French National Centre of Scientific Research (CNRS) in 1997 for his contributions to the unraveling of the brain's visual pathways. In 1999 he was appointed Director of the CNRS Centre for Brain Research and Cognition at the Université Paul Sabatier in Toulouse.



A New Instrument for the Early Diagnosis of Glaucoma

By the early 1980s the laboratories at the JCSMR had unearthed the cell types contributing to the different streams passing from the retina up into the primary visual area of the cerebral cortex of the cat and the monkey. At the same time Professor Liam Burke, of Sydney University, had shown that it was possible to selectively knock out one of these streams, that composed of large fibres, by applying pressure to the optic nerve.

Believing that elevated eye pressure in glaucoma would similarly degrade the performance of the large fibre stream Ted Maddess and Henry decided to compare the performance of this stream in human subjects with and without glaucoma, a major cause of blindness around the world. Maddess, who had previously worked mainly on insect vision, soon came up with a method to test the integrity of the large fibre pathway in humans. Based on the observation that the measured response in this pathway was not directly proportional to the strength

of its visual input, he was able to isolate the contribution from the large fibres and then to test their threshold, i.e., he measured the brightness of a test pattern at which the pathway just started functioning. As anticipated, this threshold value was significantly higher in subjects with glaucoma, apparently because of damage caused to the larger fibres.

After many clinical tests, an agreement was drawn up with an American instrument company to manufacture a new clinical instrument called the Frequency Double Perimeter. The term 'frequency double' comes from an illusion, attributed to the large fibre stream, that results in seeing twice the number of bars in a coarse grating when it is flashed on and off rapidly. The instrument has proved particularly valuable in the early detection of glaucoma, since not only are the large fibres the first to suffer damage, but they also make such a small contribution to acute vision that their absence results in a barely noticeable visual loss.

A number of clinical evaluations from

around the world have now supported the experimental findings of the new instrument, and it has become an essential part of

the clinical armory for assessing the health of the large fibre stream.



Ted Maddess (1956–) completed his undergraduate training at the University of British Columbia and his PhD (1985) and a year of postdoctoral study in the Research School of Biological Sciences (RSBS). During this time he investigated temporal adaptation in the visual responses recorded from the insect eye. He worked as a Postdoctoral Fellow in the JCSMR from 1986 to 1989, funded by a Canadian Medical Research Council Fellowship. With Geoff Henry, he followed a new line of research that led to develop a clinical method for the early detection of glaucoma. For this work, which resulted in the commercial production of a highly successful instrument, he received the Rimpac Award for innovative research in Australian science and the Australian Technology Prize. On leaving the JCSMR, he reactivated his Postdoctoral Fellowship with RSBS and was appointed a Research Fellow in 1995 and a Fellow in 1999. He is currently the head of the Biotechnology Transfer Unit in RSBS.

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Functions of the Supplementary Motor Area of the Primate Cerebral Cortex

by Robert Porter

In 1951, the Canadian neurosurgeon Wilder Penfield and his associates in Montreal, by stimulation of the human brain in patients undergoing neurosurgery, identified a region in front of the classical 'motor' cortex from which complex movements and the assumption of postures could be produced. In the assumption of postures, 'the musculature of both sides of the body is employed

appropriately to bring about the change in position'. To understand more about the neurophysiology of this 'supplementary motor area' of the cerebral cortex, in 1979 Cobie Brinkman and Robert Porter (see p. 118) at Monash University had recorded the discharges of individual neurones in this region of the brain in conscious cooperating monkeys while the animals performed a

number of movement tasks for which they had been trained. In contrast to neurones in the motor cortex, a large proportion of those in the supplementary cortex discharged impulses in association with movement whether the task was performed by the left or the right hand. Furthermore, these discharges occurred before the motor performance and appeared to be related to the same muscular actions on each side.

Later, the potential significance of these observations was highlighted when P.E. Roland and his associates in Copenhagen studied regional cerebral blood flow in human subjects performing a learned sequence of finger-thumb touching movements. Repetition of the learned movements using one hand was associated with an increase in blood flow in the 'hand' region of the contralateral sensorimotor cerebral cortex. In addition there was an increase in blood flow in the vicinity of the supplementary motor area bilaterally. If, without making a movement, the subjects thought through the sequence of movements by rehearsing them in their heads, the blood flow again increased in both supplementary motor areas but not in the sensorimotor cortex. This bilateral association was also demonstrated to occur when the skilled movements were those accompanying speech or movements of the feet. Thus, the discharges of supplementary cortical neurones recorded in monkeys seemed likely to be a correlate of the preparation and sequencing of instructions which this area then delivered to sensorimotor cortical neurones if skilled movements were to be executed.

Moving to the JCSMR Experimental Neurology Unit in 1980, Brinkman then conducted a detailed re-evaluation of the influence of the supplementary motor cortex

on movement performance by unilaterally removing it, and subsequently assessing the use of the hand in the production of delicate and precisely controlled movement of the fingers. For a few days post-operatively, monkeys exhibited a bilateral clumsiness of finger movements used in a food collection task. There was a disintegration of the precise ordering of a whole range of postural adjustments and finger movements, which resolved after a few days and could no longer be detected.

The performance of a task that required coordination of both hands, however, was permanently impaired by unilateral removal of the supplementary motor cortex. Monkeys had learned to retrieve food pellets from holes in a perspex board by pushing a finger of one hand from above and catching the pellet in the other hand cupped beneath the board. This performance required the use of the two hands to engage in different postures simultaneously and to cooperate in sharing in components of the food retrieval task. Even with full visual control, monkeys with only one intact supplementary motor cortex made frequent mistakes. A characteristic mistake was to use the hand normally cupped beneath the hole to push from above and to simultaneously push from below with a similar action of the other hand. Instead of cooperating and sharing the task by performing different actions, both hands appeared to be in receipt of the same instructions, presumably from the single intact supplementary motor area.

This investigation, and later clinical observations in humans, established that the primate supplementary motor cortex has an important role in organising the preparation of motor programs for bilaterally coordinated skilled motor tasks.

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Development of the Single Electrode Voltage Clamp

by *Stephen Redman*

Biophysical analyses of the ionic fluxes underlying action and synaptic potentials of excitable cells have been based on the voltage dependence of these events over a range of membrane potentials. For large cells it may be possible to use one intracellular microelectrode to record action or synaptic potentials and another to pass electrical currents through the membrane, so 'clamping' its potential at preselected voltage levels. Such a voltage-clamp technique enables direct measurement of the ionic currents responsible for action or synaptic potentials, and the dependence of these currents on membrane potential provides clues as to the nature of the ions involved. Single microelectrodes can also be used, particularly for small cells, provided that a very rapid switching rate can be achieved between the recording and current passing mode.

This research had its origins at Monash

University in 1980, when Stephen Redman (see p. 135) and David Hirst (see p. 438) tried to voltage clamp smooth muscle cells using a conventional two electrode approach. To maintain two intracellular electrodes in close proximity in a smooth muscle cell proved very difficult to achieve. As a consequence Alan Finkel, then a PhD student, and Redman began discussing ideas on how to make a single electrode voltage clamp with sufficient bandwidth to be useful for clamping fast synaptic potentials in neurones. A switched single electrode clamp design had previously been published by others, but its switching rate was too slow to be useful for clamping fast synaptic events. When a current pulse is applied to the electrode, the resulting voltage across the cell membrane cannot be reliably measured until the electrode capacitance, charged by the current pulse, has dis-



Alan Simon Finkel (1954–) received the degrees of BE and PhD from Monash University, the latter in 1981 for research covering electrical transmission and synaptic activity in brain tissue. For two years he conducted postdoctoral research in the JCSMR Experimental Neurology Unit, and in 1983 moved to San Francisco to establish Axon Instruments Inc. He was instrumental in the development of all aspects of the products of the company, which grew rapidly to become a major manufacturer of instruments and software for cellular neurophysiological research. Axon is also now becoming established in genomics and high-throughput drug screening. In 1987 Finkel returned to Australia where in Melbourne he continues his roles of full-time Chief Executive Officer and Vice-President of Research and Development of Axon. In 1994, under his guidance, Axon founded Optiscan Pty. Ltd., an Australian company dedicated to the development of ultrahigh-magnification endoscopes for imaging individual cells in the human body. Finkel is currently a member of the Academic Advisory Committee of the Electrical and Computer Systems Engineering Department of Monash University and the Medical Research Board of the JCSMR.

charged. The first ideas tried at Monash to speed up the discharge process turned out to be impractical.

Finkel and Redman moved to the JCSMR Experimental Neurology Unit in September 1981 and this project became the top priority. The final successful design relied on a theoretical analysis of the circuit conditions throughout the switching cycle, including the cellular contributions, careful design of the electronic circuits, and fabrication of the intracellular electrodes to minimize their capacitance. The theoretical analysis provided a clear understanding of the correct way to use the instrument and to avoid measurement artefacts.

The prototype clamp was used to measure synaptic currents evoked in spinal motoneurons *in vivo* by stimulating single Group 1a afferent axons. Only synaptic connections made on the soma were suitable for voltage clamping, as these provided an undistorted record of the synaptic current. The synaptic potentials evoked at these synapses were recognized by their time course, having the fastest rise and decay

times of all synaptic potentials evoked in motoneurons. As somatic connections were found very infrequently, it took many experiments to accumulate a few recordings. These measurements were the first ever made of a synaptic current in a mammalian central neurone, and were used to derive the open time of the excitatory synaptic channel, its voltage dependence and to unequivocally demonstrate a reversal potential of 0mV.

Finkel then collaborated with Hirst and van Helden of the Department of Pharmacology to measure the synaptic current evoked in arterial smooth muscle by stimulation of sympathetic nerves (see p. 436). Again, this was the first time a synaptic current had been measured in smooth muscle. The international neuroscience community rapidly learned about this fast single-electrode clamp suitable for measurements on central nervous system neurones, and appropriate equipment soon became available commercially when Finkel moved to California in 1983 and established Axon Instruments Inc.

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Quantal Analysis of Synaptic Transmission in Central Neurones

by Stephen Redman

A characteristic morphological feature of chemical synapses is the presence of vesicular organelles within the presynaptic terminal. These vesicles contain the chemical transmitter of the particular synapse, and the process of neurotransmission involves the release of the contents of a vesicle into the

synaptic cleft, rapid diffusion of transmitter molecules to activate specific postsynaptic receptors and the opening of ion channels characteristic of the type of synapse. The resultant ionic current modifies the resting potential and the excitability of the postsynaptic membrane.

Quantal analysis is a statistical procedure used to identify the 'quantum' of synaptic transmission, i.e., the current (or voltage) generated by the release of transmitter from a single synaptic vesicle. Quantal analysis can also provide information on the number of sites at which transmitter is being released, and the probability of transmitter release at these sites. The method relies heavily on quantisation of the synaptic currents recorded in response to repeated activation of a synaptic connection, i.e., the amplitudes must be distributed as a series of peaks and troughs, with the separation of the peaks corresponding to the quantal response.

While at Monash University, Stephen Redman, in collaboration with Bruce Walmley, Frank Edwards and Julian Jack, developed a method to extract the quantal parameters at the excitatory synapses between a single Group Ia afferent fibre and spinal motoneurons *in vivo*. The method was based on deconvolving the measured amplitude distribution of an intracellularly recorded excitatory postsynaptic potential (EPSP) with the baseline noise for that EPSP. This procedure worked well for these particular synapses, where the quantal response was usually several times larger than the recording noise. When this method, however, was applied later in Canberra by Redman and Christian Stricker to evoked excitatory responses in the hippocampal cortex *in vitro*, the quantum to noise ratio was smaller than that found in

the spinal cord *in vivo* and the method was open to criticism.

Stronger statistical tests for quantisation were required, and the collaboration of Daryl Daley from the Centre for Mathematics and its Applications in the ANU School of Mathematical Sciences was sought. Daley showed how the Wilks criterion could be used to establish an objective statistical test for quantisation within specified confidence limits. If quantisation was present, it was then possible to use the Wilks criteria to extract the quantal parameters from the amplitude distribution of the synaptic responses. Finally, bootstrap techniques were used to obtain confidence limits for these quantal parameters. These procedures established a degree of statistical rigor to quantal analysis which had previously been absent.

These new methods were applied to synaptic transmission in the hippocampus, between Schaffer collaterals and CA1 pyramidal cells. Quantal parameters were obtained from control recordings, and from recordings taken after conditioning stimulation which enhanced the synaptic strength (long-term potentiation). Stricker, Redman and Anne Field showed that long-term potentiation is caused partly by an increase in the size of the quantal current evoked at excitatory postsynaptic receptors, together with an increase in the average number of quanta in each response. The mechanisms causing the increased number of quanta are still unclear.

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A Persistent Membrane Sodium Channel

by Peter Gage

Research which was initiated at the University of New South Wales by Peter Gage (see p. 133) and his colleagues, prior to his taking up appointment as Professor and Head of the Department of Physiology JCSMR in 1984, is providing insights into the cause and possible reduction or prevention of ischaemic cell damage that occurs during a heart attack or a stroke. Using hippocampal slice techniques developed in his laboratory at the University of New South Wales, together with single electrode voltage clamp techniques developed at Monash University in Stephen Redman's laboratory (see p. 453), Gage and Christopher French, a PhD student, studied voltage-activated currents in pyramidal neurones in hippocampal slices. An effect of the sodium channel blocker tetrodotoxin (TTX) suggested that hippocampal neurones had an unusual persistent sodium current which was not inactivating during prolonged depolarizations.

This project was taken up in Canberra in 1984 by Pankaj Sah, a PhD student, and Postdoctoral Fellows Alastair Gibb and Kevin Buckett, who for the first time completely characterized these persistent sodium currents using improved patch clamp techniques. Another Postdoctoral Fellow, Graham Lamb, used the vaseline gap technique to demonstrate a persistent sodium current in voltage-clamped skeletal muscle. Results using denervated striated muscle suggested that denervation fibrillation was due to the persistent sodium current. Subsequently, similar currents were recorded from cardiac muscle by Yue-kun Ju, a PhD student, and David Saint, a Postdoctoral Fellow.

These exciting basic scientific findings related to a small sodium current very resistant to inactivation had not been described in the earlier classical papers of Hodgkin and Huxley, probably because of its small size. This current certainly has a role in the repet-

itive action potentials of neurones generated during small depolarizations such as excitatory synaptic potentials, and may also boost transmission along neuronal dendrites. Gage and his collaborators then became aware of papers claiming that drugs blocking membrane sodium channels could reduce cell damage produced by hypoxia. These observations, however, had received little attention since classical, TTX-sensitive, sodium channels were considered to become inactivated during hypoxia-induced depolarization, and were thus unlikely to be involved in cell damage. Further experiments using cardiac muscle and neurones, however, showed that the persistent sodium current was actually boosted by hypoxia.

Thus one of the first events leading to cell damage during ischaemia was uncovered serendipitously. When arteries in the heart are blocked, heart cells deprived of oxygen are damaged and patients often die as a consequence of irregular heart contractions (arrhythmias). Eventually, hypoxia kills heart cells, so reducing the heart's pumping efficiency and leading to congestive heart failure. Similarly in the brain, strokes are the result of blocked arteries or bleeding which deprives neurones of oxygen and kills them. For some time it has been known the cells deprived of oxygen are killed by an intracellular accumulation of calcium. During hypoxia calcium may enter cells because of increased calcium channel activity or through channels activated by abnormally high extracellular levels of glutamate. Clinical trials, however, have shown that drugs which block calcium channels or glutamate-activated channels are not very effective in treating stroke patients.

There is evidence that cellular accumulation of calcium during hypoxia can be blocked by preventing the entry of sodium ions, and Gage and his colleagues believe

that calcium accumulation depends on the increased activity of persistent sodium channels. Current research is focussing on the link between hypoxia and increased persistent sodium channel activity, in order to

find a selective method for blocking these channels. Suppression of this sodium entry may provide a new method for reducing cell damage during heart attacks and strokes, so improving the prognosis for patients.

Further reading

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Muscle Research at the JCSMR

by Angela Dulhunty

Research into skeletal muscle physiology began at the JCSMR in 1958 with innovative work by Arthur Buller, John Eccles and Rosamond Eccles on cross-innervation which showed that the central nervous system was responsible for determining whether a skeletal muscle was a fast-twitch dynamic muscle or a slow-twitch tonic muscle. Russell Close, appointed a Research Fellow in 1960, followed Eccles' lead in studying fast and slow twitch muscle. The emphasis of his research was more the dynamic properties of the muscles, rather than their regulation by innervation, and he developed methods for studying intracellular calcium transients in single muscle fibres.

Angela Dulhunty joined the Department of Physiology in 1984 and, although she did not collaborate with Close, they had common interests in the properties of fast- and slow-twitch muscle. Her interests then and now have been focussed on the process of excitation-contraction coupling, which is the mechanism responsible for turning the muscle surface membrane action-potential into calcium release from internal stores in the muscle's sarcoplasmic reticulum. Mus-

cle contraction depends entirely on that release of calcium ions.

The activities of the Muscle Research Group from 1984 to 1998 evolved with the introduction of new techniques and the discovery of key proteins in the molecular transduction of the action potential into calcium release from the sarcoplasmic reticulum. In the early 1980s little was known about the mechanism of excitation-contraction coupling or the nature of the molecules involved in the process. In 1984 the group completed the first, technically challenging, studies of asymmetric charge movement in mammalian muscle. These studies required the insertion of three microelectrodes in close proximity within 50µm of the tendon end of a muscle fibre, and then sophisticated analyses of recorded electrical signals. The experiments revealed minute electrical currents generated by the movement of parts of voltage sensitive molecules through the membrane in response to a change in membrane potential. These minute electrical signals were the first step in the process of excitation-contraction coupling.

In 1987 experiments were completed which led to the confident prediction that

the dihydropyridine receptor calcium release channel was the voltage sensor for excitation-contraction coupling, and that the movement of parts of the protein through the membrane generated the charge movement recorded earlier. Our predictions were confirmed in 1989 when Beam and colleagues showed that deletion of the dihydropyridine receptor abolished excitation-contraction coupling. During the same period (1984–1987) members of the Group, using a combination of freeze-fracture and thin section electron microscopy, identified novel structures in the triad junction that were associated with excitation-contraction coupling. The triad junction in muscle separates the surface/T-tubule membrane and the sarcoplasmic reticulum. The T-tubule membrane of the triad contains the dihydropyridine receptor voltage sensor, while the adjacent sarcoplasmic reticulum membrane contains the calcium release channel which allows calcium to flow from the lumen of the reticulum following the surface action potential. Structures were identified that were continuous between T-tubule and sarcoplasmic reticulum membranes, and presumably represented a macromolecular complex containing the dihydropyridine receptor and the calcium release channel.

In the late 1980s the calcium release channel in the sarcoplasmic reticulum was identified and purified, largely due to its high affinity for the plant alkaloid ryanodine, hence the protein became known as the 'ryanodine receptor'. The Group quickly developed the lipid bilayer technique for recording single channel activity from ion channels in the sarcoplasmic reticulum including ryanodine receptors. Discoveries during the 1990s have been related firstly to the regulation of ryanodine receptor by cytoplasmic factors and by parts of the dihydropyridine receptor and secondly to anion channels in the sarcoplasmic reticulum membrane.

The first studies were devoted to the regulation of the ryanodine receptor channel activity by calcium and magnesium ions. Activation of the ryanodine receptor by

Ca^{2+} is the basis of excitation contraction coupling in cardiac muscle, while inhibition by magnesium is important in suppressing skeletal ryanodine receptor activity when the muscle is not contracting. The findings showed clearly for the first time how the Ca^{2+} activation and Mg^{2+} inhibition sites differed in skeletal and cardiac muscle, and that two different sites on the protein were required for Ca^{2+} activation and Mg^{2+} inhibition sites. At the same time other members of the Group were conducting ground-breaking work into the effects of sulphhydryl modification on the activity of the ion channel, and others were studying the all important regulation of the channel by the FK506 binding protein FKBP-12. FKBP-12 regulates the co-ordinated opening of the ryanodine receptor channel by the four subunits of the protein and is important in excitation-contraction coupling.

Three types of anion channels were found in the sarcoplasmic reticulum membrane. The first, a channel identical to the mitochondrial voltage dependent anion channel, was rarely seen, but was resident in the sarcoplasmic reticulum and was localised to this organelle using ultrastructural techniques. A second channel, the large chloride channel, is constitutively open in bilayers and contained a myriad of conductance states. The third type of channel, the small chloride channel, is highly regulated by Ca^{2+} , by inositol phosphates, by ATP and by redox state. The small chloride channel has a high phosphate permeability, and is considered to allow phosphate movement into the sarcoplasmic reticulum and thus to be important in muscle fatigue.

In the late 1990s work continued with the ryanodine receptor ion channel, but the main focus has been on the II-III loop of the dihydropyridine receptor which is the part of the voltage sensor essential for excitation-contraction coupling. It is currently believed that a protein/protein interaction between the II-III loop of the dihydropyridine receptor and the ryanodine receptor is an essential step in excitation-contraction coupling. The conformational change in the membrane-spanning segment of dihydropy-

ride receptor (measured as asymmetric charge movement) is transmitted to the II-III loop of the protein and thus communicated to the calcium release channel. The effects of peptides corresponding to different parts of the II-III loop have been examined on ryanodine receptor channel activity, and strong activation of the channel by two distinct parts of the loop have been found. Using Nuclear Magnetic Resonance the structural requirements for activation of the ryanodine receptor by these fragments of the II-III loop have been determined.

In summary, muscle research at the JCSMR has evolved over the past 40 years from the definition of the macroscopic properties of skeletal muscle in the whole animal and its regulation by the central

nervous system, to the elucidation of excitation-contraction coupling in intact fibres, to the molecular events that occur in the muscle's membranes during excitation-contraction coupling. The molecular mechanisms involved in excitation-contraction coupling will continue to be studied into the 21st century and our insight into the processes in skeletal and cardiac muscle contraction will continue to expand. With increased knowledge of molecular structure and function new and more effective drugs are likely to be developed to combat heart failure, muscular dystrophy and other diseases leading to muscle weakness, and also drugs for terminating episodes of malignant hyperthermia.

Further Reading

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Angela Fay Dulhunty (1946–) graduated BSc from the University of Sydney in 1969 and received her PhD from the University of New South Wales in 1973 (DSc 1988). Following a two year Muscular Dystrophy Postdoctoral Fellowship at The University of Rochester Medical Centre, she was a Lecturer in the Department of Anatomy, University of Sydney (1976–1980), Senior Lecturer (1980–1984). From 1983–1984 she was a Fellow in the Nerve Muscle Research Centre at the University of New South Wales. Appointed a Fellow in the Department of Physiology, JCSMR, in 1984, Senior Fellow in the Division of Neuroscience in 1988, and Professor in 1997, she has continued her research into muscle excitation-contraction coupling as Leader of the Muscle Research Group since 1989, from August 1998 in the School's Division of Biochemistry and Molecular Biology.