Review

NEURON TO NERVE TERMINALS: ASPECTS OF NEUROPATHOLOGY AND TOXICOLOGY

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ABSTRACT

The nervous system is highly plastic. Its development and maturation is characterised by neuronal proliferation, specialisation and loss and the sorting of synaptic connectivity. Transmission (chemical and electrical), receptor and ion channel transcription and distribution all contribute to the emerging selectivity of action. Numerous factors – especially genetic and autoimmune – and extrinsic factors – synthetic and natural toxic chemicals – disrupt this process of development and maturation or interfere with the function and structure of the mature system. In this review, the response of the peripheral nervous system to such influences is discussed.

Key words: Nervous system, neuromuscular transmission, nerve terminal, neurotoxicology, neuropathology

INTRODUCTION

This review is concerned with the neurology, neuropathology and neurotoxicology of the peripheral motor nervous system. These areas of clinical and scientific interest are often treated as if they are totally self contained but the interpretation of data derived from pathology and toxicology can only be considered complete if they allow one to describe the structural and functional correlates of clinical disease. There should, therefore, be no barriers drawn between the three disciplines; each informs and is informed by the other and each should be underpinned by an adequate appreciation of basic anatomy and physiology. This review covers the organisation and function of the peripheral motor nervous system from neuron to nerve terminal and its response to inherited, autoimmune and neurotoxic disease. The intention is to explore the contribution that a multidisciplinary approach can make to our understanding of the biological basis of disorders of the peripheral nervous system and to discuss some of the current but unproven thoughts on the interaction between environmental, immunological and genetic factors in the expression of clinical disease.

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The review is particularly directed towards undergraduate and postgraduate students of basic biomedical sciences and medicine who wish to work at the science/clinical interface in multidisciplinary groups. The references have concentrated on key works, both classical and modern. All primary references will be found therein.

The nervous system

The nervous system is highly integrated and its development is characterised by programmed phases of cellular death, differentiation, migration and integration. Anything that disrupts these processes during development can have catastrophic and irreversible effects on neurological function. Neurons are metabolically active and are exquisitely sensitive to the deprivation of oxygen or glucose and to events that disrupt glycolytic metabolic pathways. Genetic and autoimmune factors may be involved in the expression of neurological disease as well as a large number of environmental chemicals, both synthetic and natural. Finally, the capacity to repair neuronal damage is limited by the very low numbers of competent neuronal stem cells in the nervous system [24].

The central nervous system is largely protected from noxious agents by the blood-brain barrier, a layer of tightly bound endothelial cells lining the blood vessels, that prevents the passage of many potentially toxic molecules into the brain. But the

barrier is not complete in many areas, including area postrema, the hypothalamus, the pineal and the ganglion cells of the dorsal afferents. These areas may be exposed to circulating toxic agents that are otherwise excluded from the brain. The blood-brain barrier is naturally leaky in the very young and the very old and these members of the population are particularly vulnerable to neurotoxic agents. The blood brain barrier may also be rendered leaky by some environmental toxic agents such as lead.

The peripheral nervous system

The peripheral nervous system comprises the sensory, motor and autonomic nervous systems. Though protected by the blood-axon barrier and the perineurium all parts of the peripheral nervous system are much more susceptible to exposure to noxious agents than is the central nervous system. There are two major reasons for this. The first relates to the extraordinary length of many peripheral axons. For example, a single neuron in the anterior horn of the spinal cord with a diameter of around 50μm may be supporting an axon 1 m long and the capacity for physical damage or exposure to toxic agents is clearly very large. Secondly, the terminals of both motor and sensory neurons are protected by neither the blood-axon barrier nor the perineurium and are thus very convenient attack points (Fig. 1). In this review the relationships between genetic, autoimmune and toxicological factors involved in disease of the peripheral motor system are explored, special attention being paid to the concept of plasticity. The motor system is used as it allows me to discuss in some depth the importance of the chemical synapse as a target for numerous important neurotoxins.

The motor unit

The structural and functional unit of the peripheral motor system is the motor unit. In the mammal a single motor neuron innervates a population of muscle fibres within a particular muscle. The motor unit comprises the single motor neuron, its axon and the population of muscle fibres it innervates. The number of muscle fibres innervated by a single motor neuron is highly variable. In muscles involved in the making of small and very finely graded changes in force the motor units incorporate very few muscle fibres and in muscles involved in the making of large and relatively crude changes in force, the motor units incorporate many muscle fibres. For example, a single motor unit in human eye muscles may comprise as few as 5-10 muscle fibres innervated by a single neuron whereas motor units in the human gastrocnemius muscle may comprise as many as 1500-2000 muscle fibres innervated by a single neuron. The individual muscle fibres comprising a motor unit are matched in terms of functional properties and thus act as part of a highly co-ordinated structure in which, in normal circumstances, all the muscle fibres of a unit contract together in response to the activation of the motor neuron. Increments in force generated by a muscle are achieved by either the progressive recruitment of additional motor units or by increasing the frequency of firing of a single motor unit [6,12,13]. The muscle fibres comprising a single motor unit are dispersed throughout the relevant muscle and this feature of organisation ensures that a change in force is evenly distributed over the insertion of the muscle.

The anatomical organisation of the motor unit

The cell bodies of the motor neurons are formed early in development and migrate from the neural epithelium to form the antero-lateral marginal zone of the developing spinal cord. In the mature cord the motor neurons are located in the anterior horn. Those motor neurons innervating an individual muscle typically occupy a defined column that extends through 2 or 3 segments of the spinal cord. Those motor neurons innervating proximal muscles are normally more medial than those innervating distal ones. The axons projecting from the anterior horns of the cord join with sensory axons of the posterior cord to form the peripheral nerve containing a mixture of motor and sensory axons supplying or projecting from a number of individual muscles. As the motor axons reach their target muscle, they form a small congregation of axons that splits from the main nerve trunk to form an intramuscular nerve that, in turn, separates into ever smaller axonal bundles. Eventually the single motor axons branch to innervate the muscle fibres comprising their particular motor units. Axonal branching is rare in the extra-muscle component of the nerve.

A typical peripheral nerve contains a mixture of both myelinated and unmyelinated axons. The former fall into two groups: large diameter axons with a diameter, in the human, of approximately 8-20 μm and those with a diameter of 1-8 μm. The large diameter axons include the α-motor axons supplying

the extrafusal muscle fibres and the sensory axons projecting from the Golgi tendon organs and the primary 1a afferents from muscle spindles. The smaller axons comprise the γ-motor axons supplying the muscle spindles and the secondary afferents projecting to the cord from the muscle spindles. The unmyelinated axons are entirely sensory in function. During normal development both axon diameter and, where appropriate, thickness of myelin (or to be more accurate, number of myelin lamellae) increase until approximately 5 yrs of age (Fig. 2). A more thorough description of the anatomy and physiology of the motor unit may be found in Slater and Harris [29].

Two particular features of the motor axon relevant to this review are myelination and the filamentous proteins, particularly neurofilaments and microtubules, that characterise the cytoskeleton of the axon. Myelin, produced by the Schwann cells acts as a lipid insulator enwrapping the axons. The insulated parts of the axon take little part in the ion movements that underpin axonal conduction. Conduction principally occurs at the nodes of Ranvier, naked portions of the axon that occur at intervals of 0.2-1.0 mm along the length of the axon where voltage gated Na^+ and K^+ channels are concentrated. Neurofilaments comprise a complex of three polypeptides forming

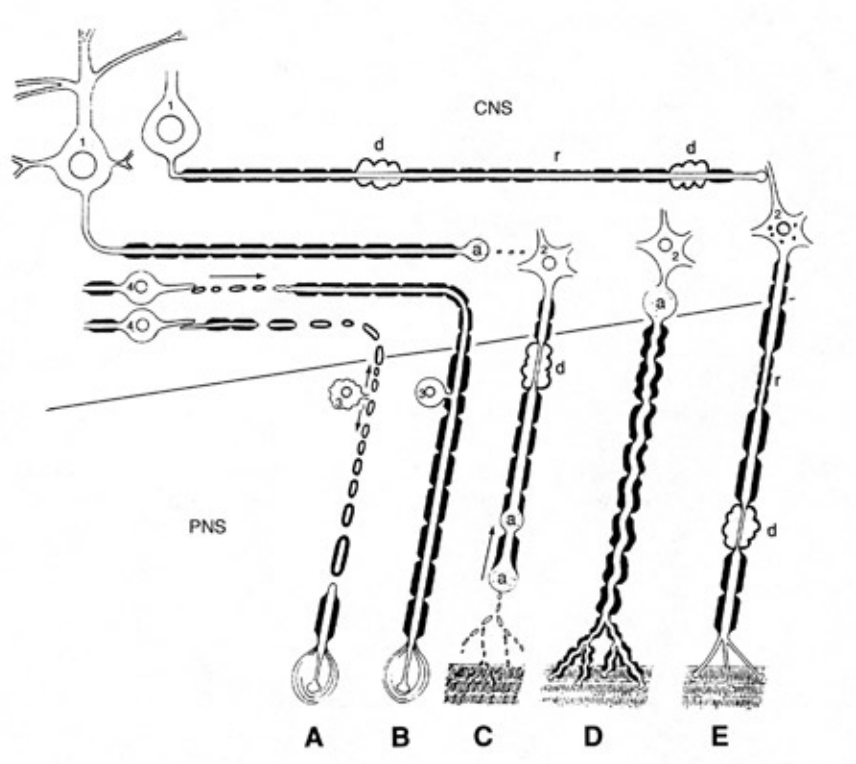


Figure 1. Cellular effects of some neurotoxic chemicals illustrated by upper (**2**) and lower (**2**) motor neurons, dorsal root ganglion cells (**3**), and second-order sensory neurons (**4**) in the gracile nucleus of the medulla oblongata. The central nervous system (**CNS**) is represented above the sloping horizontal line, the peripheral nervous system (**PNS**) below. The peripheral receptors in fibers A and B are pacinian corpuscles. Fibers C-E innervate extrafusal muscle fibers. (**A**) Neuronopathy: *Doxorubicin* irreversibly damages the neuron (**3**) resulting in a rapid anterograde (**arrows**) pattern of total axonal breakdown and myelin loss. (**B**) Central distal axonopathy: *Clioquinol* induces retrograde degeneration (**arrow**) of the central axonal process of the dorsal root ganglion cell (**3**) but leaves the cell and the peripheral process intact. (**C**) Central-peripheral distal axonopathy: *2,5-Hexanedione* induces the formation of distal neurofilament-laden axonal swellings (**a**) and retrograde axonal degeneration (**arrow**) to develop slowly in long and large central and peripheral axons. Muscle atrophy occurs unless axons regenerate and sprouts reinnervate the muscle. The anterior horn cell (**2**) is left intact and, eventually (after months to years), a secondary demyelination in the ventral root ensues (**d**). (**D**) β,β′-*Iminodipropionitrile* causes giant axonal swellings (**a**) to develop in the intraspinal portion of the axon; the distal axon attenuates but does not degenerate. (**E**) Myelinopathy: *Acetylethyltetramethyltetralin* secondarily causes myelin bubbling (**d**) focally along large-diameter central and peripheral nerve fibers. Axonal denudation is followed by remyelination (**r**): this occurs in the ventral roots and medulla oblongata when demyelination (**d**) is in progress in the peripheral nerve and elsewhere. A similar process takes place in a primary disease of the myelinating cell, except that remyelination might not occur during chemical exposure (from Spencer *et al.*, [31]).

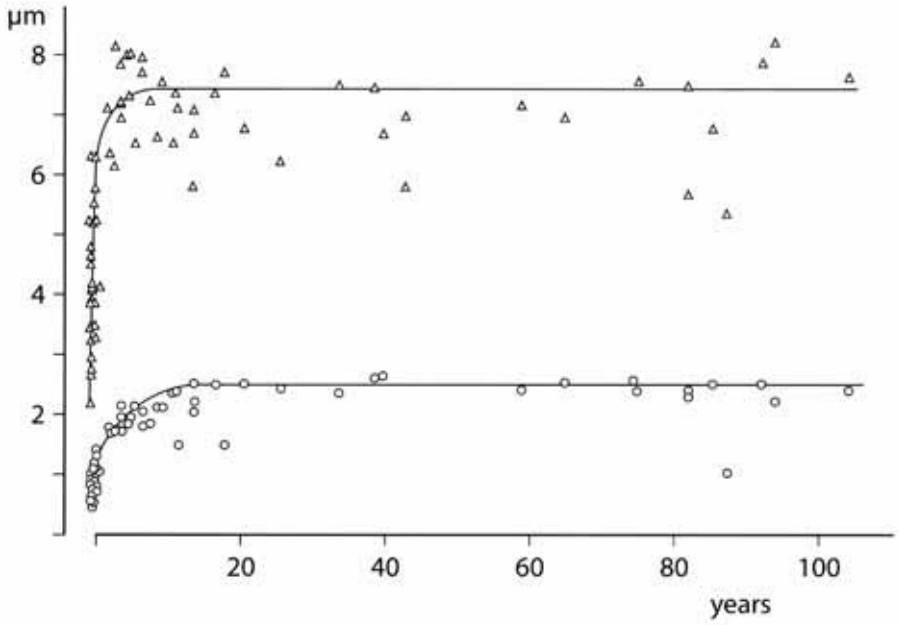
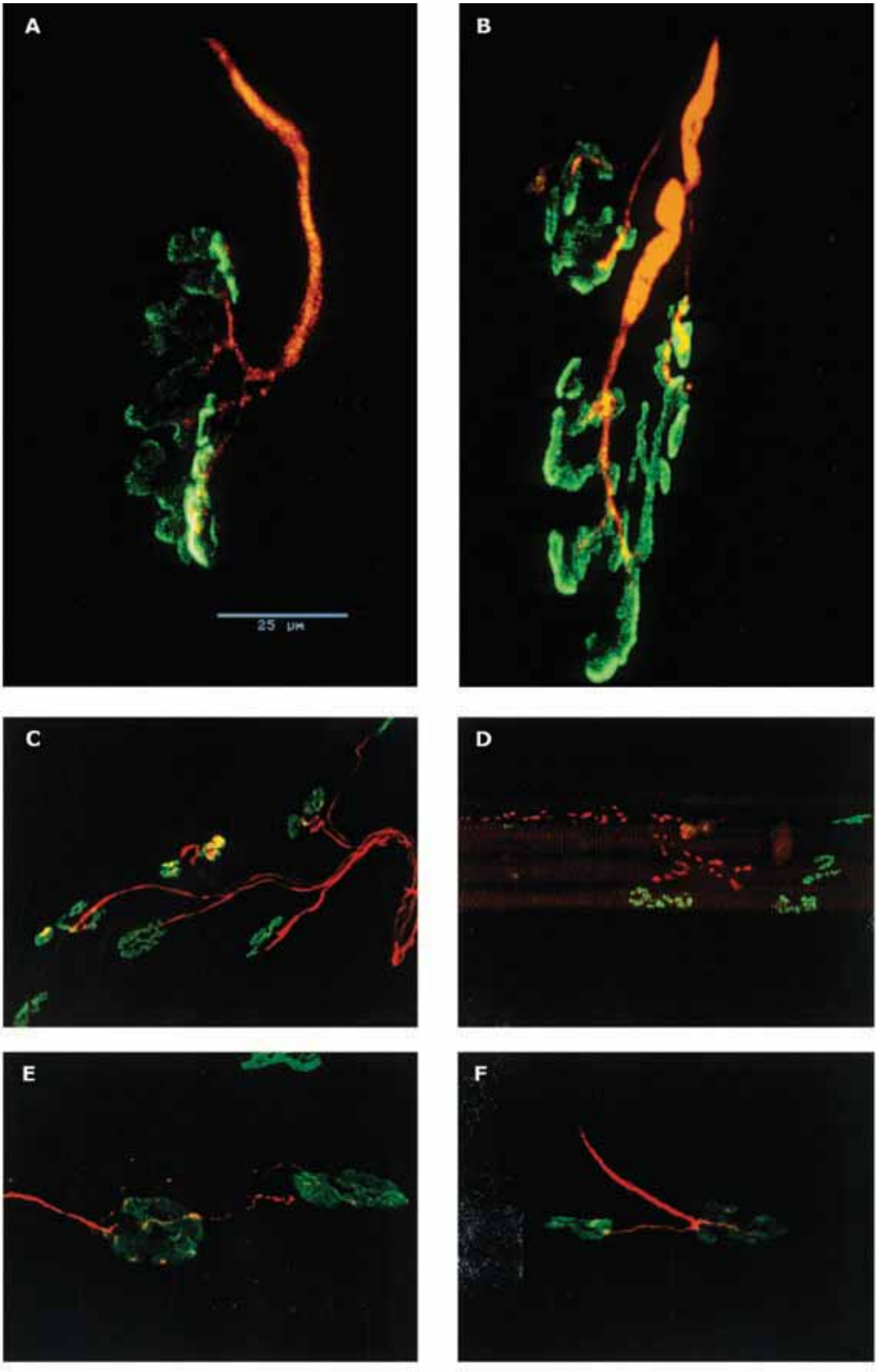


Figure 2. Development of axonal diameter (Δ) and myelin sheath thickness (O) in the sural nerve of man (biopsy and autopsy cases). (From Schröeder [27]).

a flexible rod that extends from cell body to the terminal boutons. Neurofilaments are approximately 10-15 nm in diameter and are responsible for slow axon transport, principally facilitating the movement of cytoskeletal and cytosolic proteins. Fast axonal transport of membrane bound organelles is facilitated by microtubules, structures of large diameter (approximately 20 nm) formed by the polymerisation of tubulin.

Within approximately 20 µm of its point of termination on the skeletal muscle fibre, the motor axon loses its sheath of myelin and forms a network of fine branches at the neuromuscular junction (Fig. 3A). This is the site at which information is transmitted from neuron to muscle fibre. In a typical mammalian muscle fibre the junction is located in the centre of the muscle fibre, equidistant from both origin and insertion. The unmyelinated axonal projections swell to form the terminal boutons, each of which lies in a deeply folded trough on the plasma membrane of the muscle fibre. The plasma membrane of the nerve terminal is separated from the plasma membrane of the muscle fibre by a gap of approximately 50 nm, to form the synaptic cleft. The cleft contains a layer of basal lamina, major components of which are acetylcholinesterase and agrin. The terminal is capped by the terminal Schwann cell and the entire structure is enclosed in overarching fibroblast-like cells (Fig. 4A). The terminal bouton contains large numbers of mitochondria and synaptic vesicles filled with neurotransmitter (ACh). The synaptic vesicles are 30-60 nm in diameter and inserted into the vesicle membrane are numerous specialised proteins including transmitter transporters, and proteins involved in vesicle storage, mobilisation, tethering, fusion and recycling such as synapsin,

Figure 3. Neuromuscular junctions in soleus muscles of the rat. The control (**A**) shows a single axon innervating the end plate ACh receptors. Note the fine spray of terminal axons emerging from the unmyelinated portion of the axon. In muscle (**B**), 5 days after the axon had regenerated following exposure to a neurotoxic phospholipase A_2 (β-bungarotoxin) sprouts emerge from nodes of Ranvier to innervate three individual muscle fibres in close proximity to each other. **C** - **F** show low power images of longitudinal sections of rat soleus muscle fibres. The control preparation (**C**) shows typical innervation patterns of a mammalian neuromuscular junction. Exposure to a neurotoxic phospholipase A_2 (β-bungarotoxin) caused the disaggregation of neurofilament protein and the denervation of the muscle (**D**). Regeneration (**E**, **F**) was associated with extensive nerve terminal sprouting and the development of aberrant patters of innervation. Axons labelled with anti-neurofilament antibodies, ACh receptors with FITC-conjugated α-bungarotoxin.


A
25 µm
B
C
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synaptobrevin and synaptotagmin. A third key protein, synaptophysin, has a role that is not properly understood, but it is widely used as a marker of synapses and synaptic vesicles. Synapsin is involved in the tethering of vesicles to f-actin in the nerve terminal, and these vesicles constitute a reserve pool. The plasma membrane of the nerve terminal accommodates similarly specialised proteins including voltage gated Ca^{2+} channels, transporters of choline-a precursor for ACh synthesis - and proteins involved in the docking and fusion of synaptic vesicles such as SNAP-25 and syntaxin. The process of docking takes place at specialised release sites in the nerve terminal plasma membrane and involves the formation of a complex between the SNARE protein of the synaptic vesicles (v-SNARE), synaptobrevin, and its counterparts on the terminal plasma membrane (t-SNARE proteins), SNAP-25 and syntaxin, to form a SNARE pin complex. The synaptic membrane of the muscle fibre is deeply folded and similarly specialised: the ACh receptors are found at the top of the synaptic folds in close apposition to the release sites on the nerve terminal membrane and the voltage gated Na^{+} channels are located in the depths of the folds

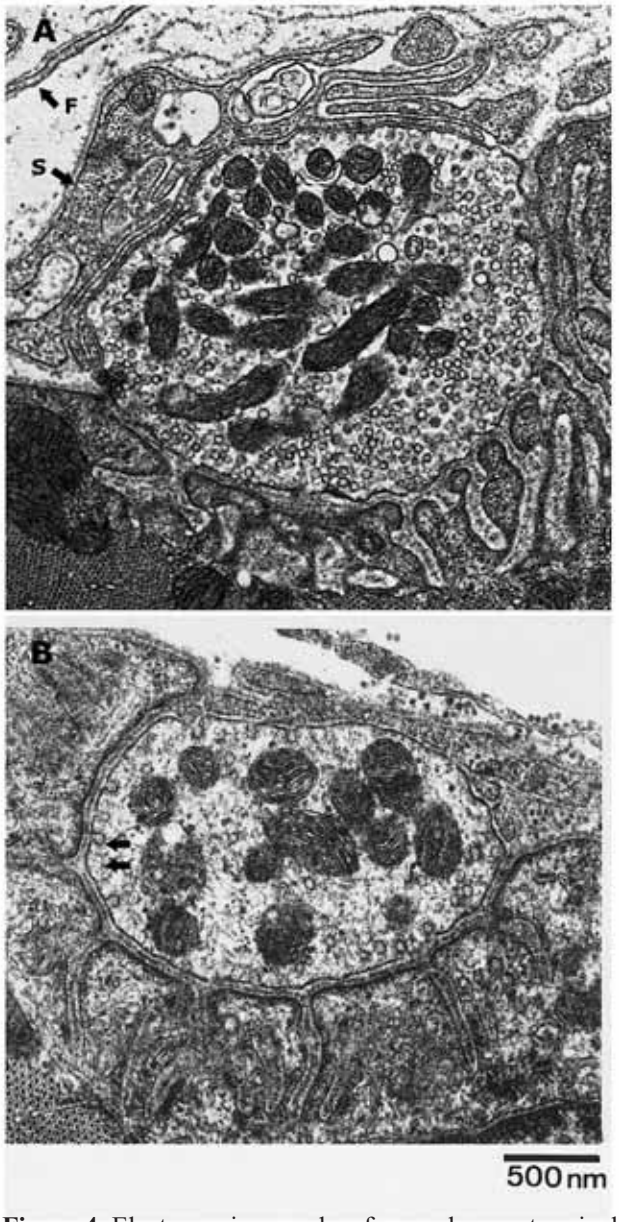

Figure 4. Electron micrographs of normal nerve terminal on a rat neuromuscular junction (**A**) and (**B**) 24 hours after exposure to a neurotoxic phospholipase A_2. Note the loss of synaptic vesicles in the latter. A few vesicles remain fused to the nerve terminal membrane (**arrow**) in (**B**). Note in (**A**) the Schwann cell (**S**) and a portion of a fibroblast (**F**). The depletion of synaptic vesicles appears to be more common than often thought. It is seen in autoimmune diseases such as Miller – Fisher syndrome and after exposure to many neurotoxic agents including organophosphates and acrylamide.

The development of the neuromuscular junction begins early in foetal development, when growing axons approach the nascent muscle fibres. The foetal muscles express ACh receptors over the entire muscle fibre plasma membrane. The axons secrete two chemical agents, agrin and neuregulin. Neuregulin activates the upregulation and transcription of ACh receptor genes in the muscle fibre nuclei at the nascent junction and agrin regulates the localisation of the receptor to the junctional plasma membrane of the muscle fibres. As the junction matures the transcription of ACh receptors outside the junctional region is reduced. The actions of agrin are mediated by rapsyn and a muscle specific kinase (MUSK), the activity of which is downregulated once the neuromuscular junction is formed and stable. The ACh receptor is a pentamer of homologous proteins. The immature form of the receptor comprises 2 x alpha subunits and one each of beta, delta and epsilon. As the neuromuscular junction matures the expression of the epsilon subunit is downregulated and the expression of the mature gamma subunit is upregulated. The process is reversed if the muscle fibre is denervated and re-capitulated during the regeneration of damaged muscle and the reinnervation of denervated muscle. Precisely how the site of innervation of mammalian muscle fibres becomes routinely located equidistant from the origin and insertion is controversial and probably involves a combination of electrical and

chemical signalling and mechanical activity induced by the functional junctions. When first formed each end-plate on the muscle fibre is contacted by axonal branches from several neurons. This stage of polyneuronal innervation is transient. As a result of competition between axons, redundant axonal branches are eliminated, motor neurons incapable of forming stable contacts with muscle fibres die and so the mature pattern of one axonal branch per muscle fibre is reached (Fig. 3C). The loss of motor neurons may involve 50% of the motor neurons first formed, but once this stage of development is over, motor neuron numbers remain stable until the stage of age-related neuronal cell death. In the human, the age-related loss of motor neurons begins at around 60 years [3].

The muscle fibre is a multinucleated cell derived from the fusion of numerous precursor cells, the myoblasts. The entire fibre is ensheathed in a basal lamina comprising a polymerised network of collagen IV and a mixture of proteoglycans and glycoproteins, and it is associated with an external layer of collagen fibres, the reticular lamina. Together, the basal lamina and the reticular lamina form a basement membrane. The limiting membrane of the muscle fibre is the plasma membrane (plasmalemma). The interior of the muscle fibre is characterised by the longitudinally orientated myofibrils. These are the fundamental units of a muscle fibre. They are typically 1-2 μm in diameter and extend without branching for the length of the muscle fibre. Each myofibril comprises serially repeating contractile units, the sarcomeres, and it is the precise alignment of neighbouring sarcomeres that give the muscle fibre its striated appearance (for an interesting review of muscle fibre striation see Huxley [15]). The boundaries of the sarcomere are the Z-discs. Each sarcomere is 2.0-3.0 μm long. They comprise a centrally located A-band of longitudinally orientated thick myosin filaments bounded by lighter I-bands. The I-bands traverse the Z-discs so that each sarcomere comprises two half I-bands of thin actin filaments interdigitating with one full A-band of myosin filaments. Mitochondria are found beneath the plasma membrane and between the myofibrils. A system of internal tubular systems is responsible for the initiation and completion of the cycle of contraction and relaxation. The transverse tubules penetrate the internal myofibrillar structures at the location of overlap between A/I bands. There they meet swellings (cisternae) of the longitudinal tubular system of the sarcoplasmic reticulum. At the junction of these two system is formed the triad (sometimes a dyad) of two cisternae and one transverse tubule. In brief, it would appear that a wave of depolarisation during the generation of an action potential across the plasma membrane of the muscle fibre is conveyed down the transverse tubule triggering the release of Ca^{2+} from the cisternae, which activates myosin ATPase at the A/I overlap and thus provides the energy required for shortening and the generation of force. The re-accumulation of Ca^{2+} by the sarcoplasmic reticulum initiates relaxation. The regulation of the organisation of the sarcomere is of great significance and two cytoskeletal proteins appear to be particularly important. Desmin, an intermediate filament protein, forms a reticulated collar around the Z-discs of myofibrils, interlinking both sequential Z-discs in a myofibril and the Z-discs of adjacent myofibrils. It also links the Z-discs of the most peripheral myofibrils to the plasma membrane. It plays a minimal role in myofibrillar development (when its place is taken by nestin and vimentiin) but it is directly involved in the mature muscle in the regulation of sarcomeric organisation, mechanical stability and force transduction. Titin is a large, highly elastic sarcomeric protein that is associated with sarcomeric elasticity and is particularly important for the regulation of sarcomeric integrity during shortening and lengthening. Numerous other proteins are involved in the stabilisation of the sarcomere (e.g. nebulin, α-actinin) but in terms of muscle pathology desmin and titin have probably attracted most attention because of their relatively high abundance [10].

Each motor unit has specific contractile and metabolic characteristics. The motor unit may principally utilise oxidative, glycolytic or mixed oxidative/glycolytic metabolism, and this metabolic differentiation is reflected in the contractile behaviour of the motor units: slow rates of rise of tension and fatigue resistance characterise those that rely on oxidative metabolism and fast rates of rise and fatigueability those that rely on glycolytic metabolism. The metabolic differentiation is directed by the physiological characteristics (particularly the rate and pattern of activation) of the relevant motor neuron and is developmentally regulated [34]. Most muscles comprise a mixture of motor units and since the muscle fibres of a single unit

are randomly distributed through a muscle, it follows that muscle fibres of different motor units are randomly distributed. This is a very important feature. If a motor neuron dies or axonal damage is severe, the undamaged axons sprout to re-innervate the denervated muscle fibres. Whatever the previous history of the denervated muscle fibre its metabolic properties (and hence its physiological behaviour) will adapt to the physiological characteristics of the "new" motor neuron.

This leads to enlarged motor units and the progressive formation of groups of muscle fibres of the same metabolic and physiological type belonging to the same motor unit – a phenomenon referred to as muscle fibre type grouping (Fig. 5). If the neuron dies after the formation of a motor unit comprising a coherent group of muscle fibres, the denervated muscle fibres atrophy to form a group of atrophied muscle fibres (termed group atrophy). These features are very important pathological features of neuron – and axonopathies (see later).

Neuromuscular Transmission and Excitation-Contraction Coupling

The activation of the motor neuron leads to the generation of an action potential in the axon-hillock, a region of the neuron at which voltage gated Na^+ and K^+ channels are concentrated. The action potential is propagated along the myelinated motor axon. The propagation is saltatory, involving the relevant movements of Na^+ and K^+ ions through the respective voltage gated ion channels located at the nodes of Ranvier. The invasion of the action potential into the nerve terminal leads to the depolarisation of the terminal boutons and the opening of voltage gated Ca^{2+} channels. Ca^{2+} ions enter the terminal boutons following the very high concentration gradient ($[Ca^{2+}]_i = 10^{-7}$ M; $[Ca^{2+}]_o = 10^{-3}$ M). The entry of Ca^{2+} into the terminal bouton has several effects. It triggers the phosphorylation of synapsin which liberates synaptic vesicles in the reserve pool from actin filaments, it promotes formation of the SNARE complex and, via synaptotagmin, it promotes the fusion of synaptic vesicles with the plasma membrane of the terminal bouton. The fused vesicle then opens and as a result ACh is released into the synaptic cleft. The synaptic vesicles are then recycled via a process known as endocytosis and accumulate ACh from the cytosol where choline acetyltransferase catalyses the formation of the transmitter from choline and acetyl CoA before re-engaging in the process of exo- and endocytosis. Acetycholine is released spontaneously at low levels as docked synaptic vesicles fuse and open randomly to liberate the contents of a single vesicle into the synaptic cleft. The electrical response of the muscle is a miniature end-plate potential. In response to the excitation of its motor neuron the invading nerve impulse results in the fusion of 50-100 vesicles and the generation of a muscle fibre action potential (described in a well known classic, Katz [16]).

The released ACh has two fates. Either it is hydrolysed by acetylcholine esterase located in the basal lamina of the synaptic cleft or it binds to the α-subunit of the junctional ACh receptor on the muscle

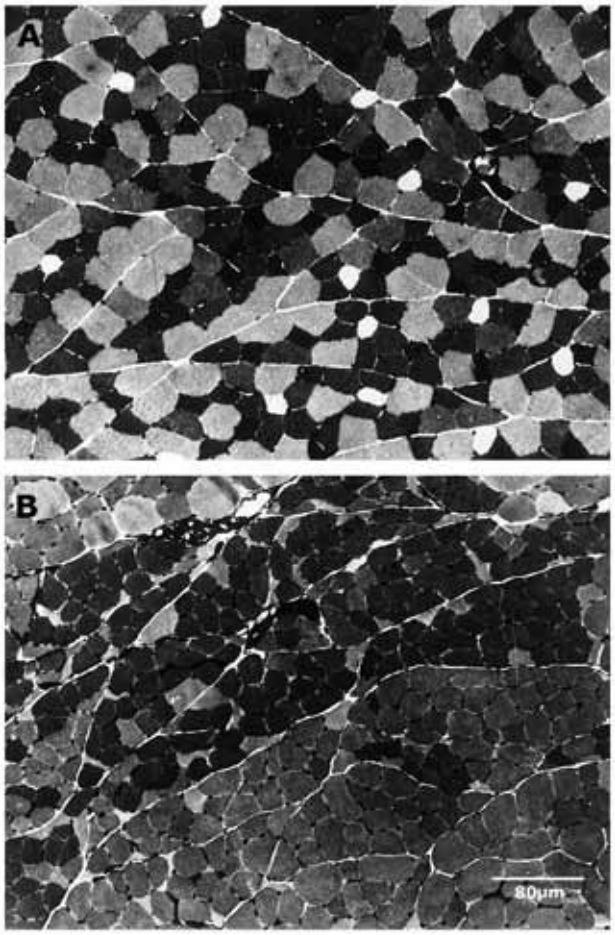

Figure 5. Extensor digitorum muscle of control mouse (**A**) and mouse with motor neuron disease (**B**). Frozen section stained for myosin ATPase activity. Dark fibres oxidative, pale fibres glycolytic. Note the random distribution of muscle fibre types in **A** and the grouping of fibre types in **B**. Note also the muscle fibre atrophy in **B**. Fibre type grouping occurs as a result of the denervation and reinnervation of muscle fibres.

fibre. This binding is transient and as soon as it is over the ACh is hydrolysed. The product of hydrolysis (choline) is taken up by the terminal bouton ready for acetylation by choline acetyltransferase in the cytosol. Depolarisation of the muscle fibre caused by the binding of ACh to the ACh receptor results from a conformational change in the receptor that, in turn, results in the opening of the receptor ionophore and the movement along their respective gradients of Na^+, K^+ and Cl^- (and possibly Ca^{2+}). The depolarisation causes the opening of voltage gated Na^+ channels in the depths of the deep folds of the neuromuscular junction and the generation of an action potential. The action potential is conducted along the muscle fibre plasma membrane and down the transverse tubules to initiate the contraction of the muscle fibre as described above. This system is immensely robust but even this, much abbreviated, outline of the organisation, structure and function of the motor unit identifies the large number of potential targets available to myo- and neurotoxic chemicals.

Plasticity in the peripheral motor nervous system

The interpretation of pathology is more effective if there is a good understanding of the different aspects of plasticity in the peripheral nervous system. Development begins with the over production of motor neurons. The loss of motor neurons during development is not random but is dependent on the muscle. Reducing the amount of muscle available for innervation results in an increase in neuronal cell death. Increasing the amount of muscle available reduces the degree of neuronal cell death. The muscle is similarly dependant upon the nerve. Muscle growth depends on innervation. Denervation during early development results in the cessation of muscle development and growth, and the permanent loss of muscle bulk if the denervation is unrepaired. Exactly similar events occur if a regenerating muscle is denervated. The muscle fails to grow and mature. In the mature animal, loss of muscle results in the much slower loss of motor neurons. The molecular basis of the interdependence of nerve and muscle, and more generally of neuronal survival and repair is not particularly well understood but certainly involves the activities of the growing number of recognised "nerve growth" and "neurotrophic" factors. There is not space here for a discussion of these factors but they have been recently reviewed [30]. Innervation patterns are also highly plastic. The polyneuronal innervation seen in the early stages of neuromuscular development is recapitulated during the regeneration of damaged motor axons or muscle fibres [8]. In cases where axonal damage affects only the terminal structures a relatively normal pattern of innervation is fully restored, but there is often a degree of fibre type grouping that results from axonal sprouts arising at nodes of Ranvier (Fig. 3B). The very large motor units that arise when there is extensive loss of neurons or motor axons are metabolically and functionally compromised and their motor neurons tend to die early, leading to grouped fibre atrophy. The motor neuron has a profound affect on the properties of the muscle fibre. The firing frequency of a motor neuron is of major significance. If a muscle is stimulated directly at a steady frequency of 10Hz (the frequency of firing of slow-type motor neurons) the muscle fibres will adopt the metabolic and functional properties of slow motor units, and this transformation involves changes in Ca^{2+} sequestration by the SR, changes in metabolism and (later) changes in the expression of myosin isoforms. The denervation of a muscle fibre leads to the reappearance of immature forms of the ACh receptor all over the muscle fibre membrane, the upregulation of the immature TTX-resistant voltage gated Na^+ channel and, of course, muscle fibre atrophy. Mechanical activity, induced by neuronal activity or by direct stimulation, causes the restriction of ACh receptor distribution, and the suppression of the epsilon subunit of the receptor and the TTX-resistant Na^+ channel. Regenerating skeletal muscle also exhibits many of the features of immature muscle. For example, during early stages of regeneration muscle fibres are supersensitive to ACh and express TTX-resistant voltage gated Na^+ channels (Fig. 6).

Age is a significant complicating factor for any study of long term disease of the peripheral nervous system. The loss of motor neurons from the age of 60 yrs [3] has already been mentioned. There is also a loss of muscle bulk and impaired neuromuscular repair, fibre type grouping and, in some muscles, a change in metabolic type.

Pathology of the peripheral nervous system

It is often convenient to compartmentalise degenerative disorders of the peripheral nervous system, and neuropathologists, neurologists and

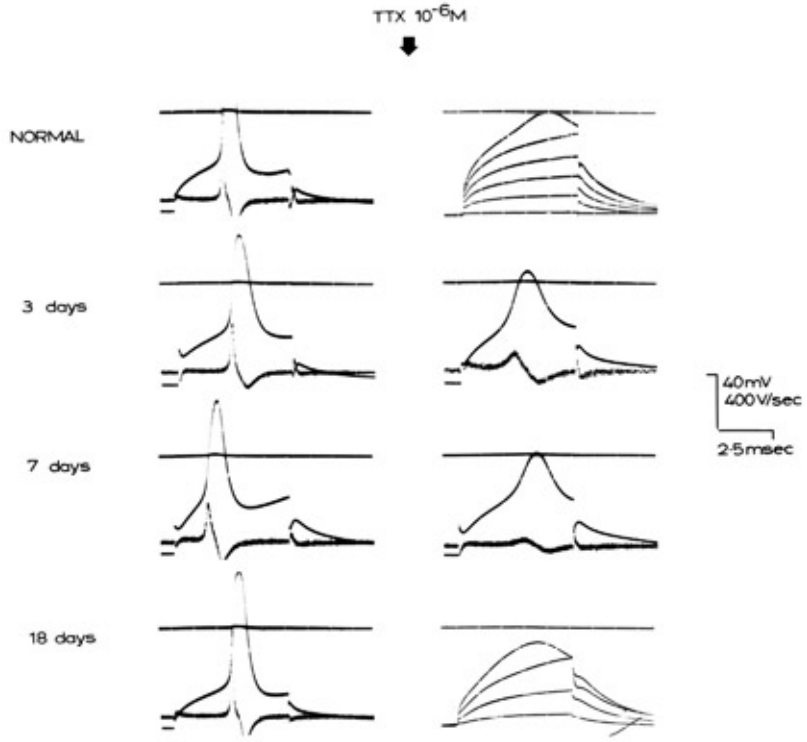

Figure 6. Action potentials generated in soleus muscle fibres exposed to tetrodotoxin (TTX). In normal muscles voltage gated Na^+ channels are blocked by TTX and no action potential can be generated. In muscles exposed to a myo- or neurotoxic phospholipase A_2 (notexin in this case) the muscle fibre produces an isoform of the voltage gated Na^+ channel that is not blocked by TTX (3 days and 7 days). As a result an action potential can be generated even in the presence of TTX. By 18 days the TTX-resistant isoform is no longer produced. The TTX-resistant isoform of the Na^+ channel is expressed in immature, regenerating and denervated muscle and can be suppressed by re-innervation or electrical stimulation. In this case, notexin caused degeneration of both nerve terminal and muscle fibre (see Grubb *et al.*, [8]).

toxicologists typically differentiate between a neuronopathy, axonopathy, disorder of neuromuscular transmission etc. But the rigid separation of neuronopathy, axonopathy, or disorder of neuromuscular transmission is not always possible as a functional or structural lesion primarily targeting the motor neuron may have secondary effects on axonal integrity and the maintenance of neuromuscular transmission (Fig. 1). Furthermore, the end result of neuronal loss, axonal degeneration or transmission failure will eventually be denervation, muscle fibre atrophy and all the other denervation-induced changes. Despite this caveat, in this review I adopt the usual convention of compartmentalisation primarily for its convenience.

Neuronopathies

The archetypal disease of the motor neurone is Motor Neurone Disease (Amyotrophic Lateral Sclerosis) a title given to a group of related diseases all associated with the primary degeneration of motor neurons. The disease has an annual incidence of around 1:100,000. It is characterised by a relentlessly developing muscular weakness and survival beyond 5 years after diagnosis is rare. The degeneration of the motor neuron leads to the dying back of the axon. Nerve terminals degenerate and the degeneration of the axons proceeds slowly back to the spinal cord. There is a loss of large diameter axons and the muscular weakness is caused by denervation of the muscle fibres. Nerve terminal sprouting, fibre type grouping and grouped atrophy in the muscles during early stages of the disease suggests that the progressive loss of motor neurons leads to a compensatory nerve terminal sprouting of surviving motor neurons and the enlargement of the motor unit but this attempt to compensate for neuronal loss is transient as surviving motor neurons succumb to this progressive disease. The biological basis of neurodegeneration is unclear.

Excitotoxicity is generally considered to be a major factor, but accelerated ageing of motor neurons and mitochondrial abnormalities are possibly involved. Around 90% of all cases are sporadic and the absence of a genetic component has led to much speculation that environmental factors might promote neuronal damage in susceptible individuals. To date no environmental factors have been identified.

Excitotoxic damage to neurons is caused by exposure to either endogenous excitatory amino acids (e.g. glutamate, aspartate) or to exogenous amino acids. For example, β-oxalyl-amino-alanine found in *Lathyrus sativa* and β-methyl-amino-alanine found in *Cycas circinalis* and domoic acid which is produced by marine dinoflagellates and accumulated by shellfish, have been implicated in severe neuronal loss when consumed [31]. The broadly accepted mechanism underlying excitotoxicity is that over excitation by the excitatory amino acids leads to the accumulation of Ca^{2+} in the neuron and a generalised loss of ion homeostasis. This leads to metabolic exhaustion as the cell attempts to restore ionic balance. At the same time mitochondria are destroyed as the organelles try to sequester the excess Ca^{2+}. The end result is the denervation of terminal structures such as skeletal muscles.

A number of toxic agents destroy neurons directly. For example, methyl mercury readily crosses the blood-brain barrier and is taken up into neurons. It is a general neurotoxin affecting a wide range of neural activities by binding to sulfhydril groups on enzymes and structural proteins, disrupting metabolism, transmitter release, mitochondrial respiration etc. Other agents (e.g. anthracyclines such as doxorubicin) illustrate different aspects of neurotoxicology. These drugs are used as cytotoxins in the treatment of a number of cancers. The drugs do not pass the blood-brain barrier and their neurotoxic effects are concentrated at those areas not protected by the barrier. Uptake into exposed neurones is rapid. The ganglia of the autonomic and peripheral sensory systems are particularly affected and the resulting ganglionopathy causes axonal degeneration and ataxia. The damage is caused by the destruction of DNA and the inhibition of DNA-repair. Vinca alkaloids (Fig. 7) are neurotoxic because they prevent the polymerisation of tubulin, leading to the breakdown of the microtubules. Cellular integrity depends on mitochondrial function, and many drugs cause neurotoxic problems because of their activity on mitochondria. The anti-viral nucleoside analogues (e.g. dideoxycytidine, dideoxyinosine) enter the neuron via nucleoside carriers and block the synthesis of mitochondrial DNA. Mitochondria in the neuron, Schwann cell and axon are all vulnerable, the relative degree of susceptibility depending on the specific nucleoside analogue involved. The result of exposure to these agents is a painful neuropathy of both motor and sensory components of the peripheral nervous system.

Axonopathies

The dying back process that occurs following the death of a neuron may also result from damage to the axon (or, of course, to simultaneous damage to both neuron or axon). Section of the motor axon leads to the very rapid loss of the terminal boutons (within 24 h) and the denervation of the muscle fibres. The loss of the distal axon is relatively delayed [28]. In due course regeneration of the distal axon results in re-innervation. If re-innervation is prevented surviving axons sprout and re-innervate the denervated muscle, at the same time expanding the size of the motor unit. The muscle then exhibits fibre type grouping. Where axonal damage is so severe that regeneration cannot occur the disconnected neuron dies. The axonal neurofilaments and the Schwann cells are also highly vulnerable to attack and are the target of a number of toxic chemicals and sources of infection. Organic solvents as a class of chemicals, and particularly the hexacarbon solvents such as n-hexane (Fig. 7) are very important neurotoxic agents. In many cases toxicity is enhanced by metabolic conversion of the solvent to a more toxic metabolite (eg. hexane is metabolised to 2-hexanone and 2,5-hexane dione (Fig. 8). The hexacarbon solvents promote the cross-linking of neurofilament proteins, the formation of neurofilament accumulations and thus the impairment of axonal transport. They also inhibit glycolytic enzyme activity that is responsible for local energy production. Acrylamide (Fig. 7) promotes neurofilament aggregations and impaired axonal transport probably by oxidising sulphydril groups to promote the cross linking of all sulphydril containing proteins. In all cases the deficits in axonal transport precede the degeneration of the axons, and in all cases the distal components of the axon are the first to be affected, terminal boutons exhibiting a loss of synaptic vesicles and then degeneration. Typically, degeneration of neurofilament leads to the accumulation of damaged

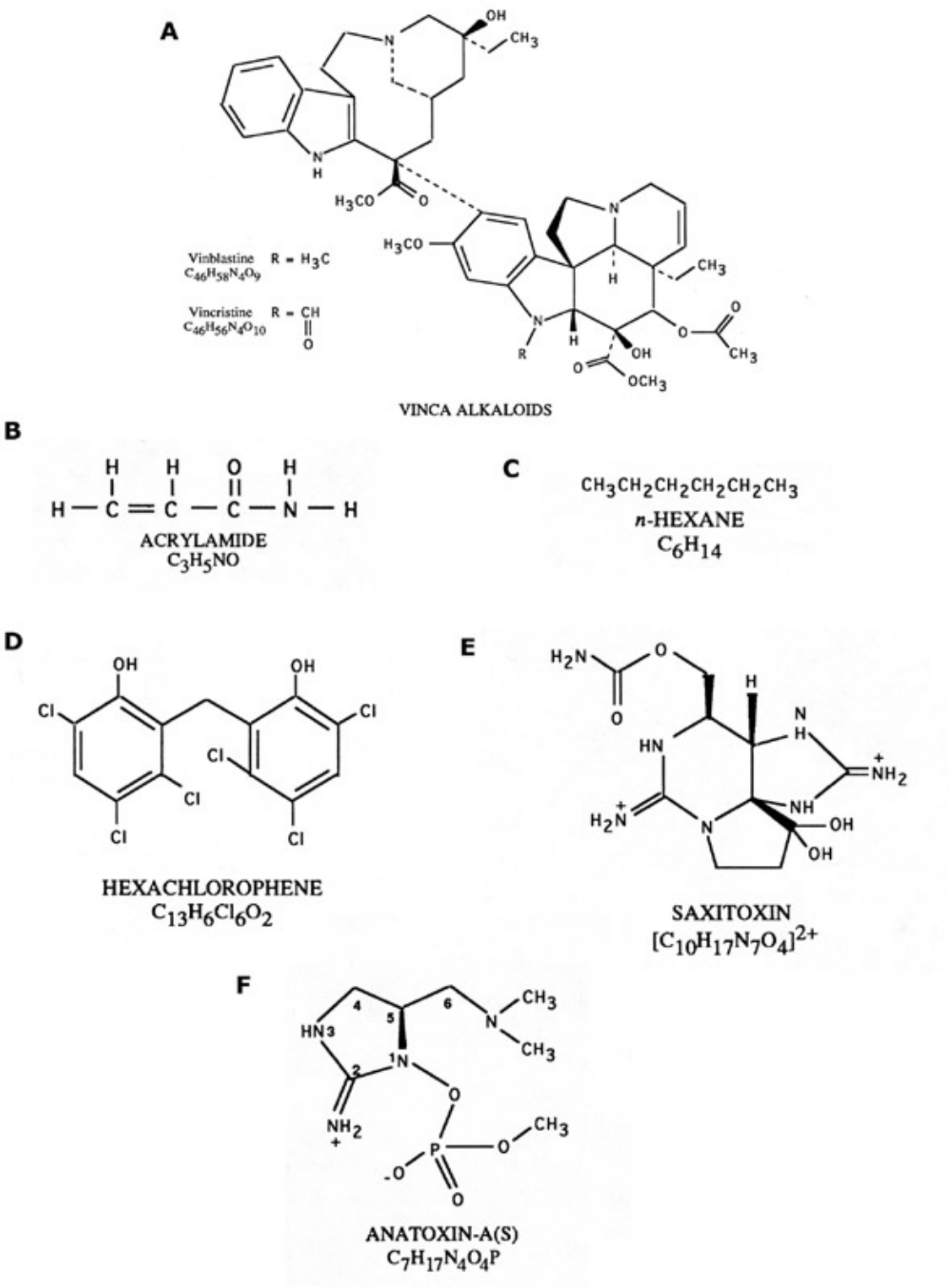

Figure 7. The diversity of chemical agents that can affect the nervous system is extensive, and so are the neural targets. Vinca alkaloids (**A**) damage neurons. Acrylamide (**B**) and N-Hexane (**C**) target axons to cause an axonopathy. Hexachlorophene (**D**) causes a myelinopathy and saxitoxin (**E**) blocks voltage gated Na^+ channel. Anatoxin-a(s) is a rare example of a naturally occurring organophosphate that blocks acetylcholinesterase activity. All from Spencer *et al.*, [31].

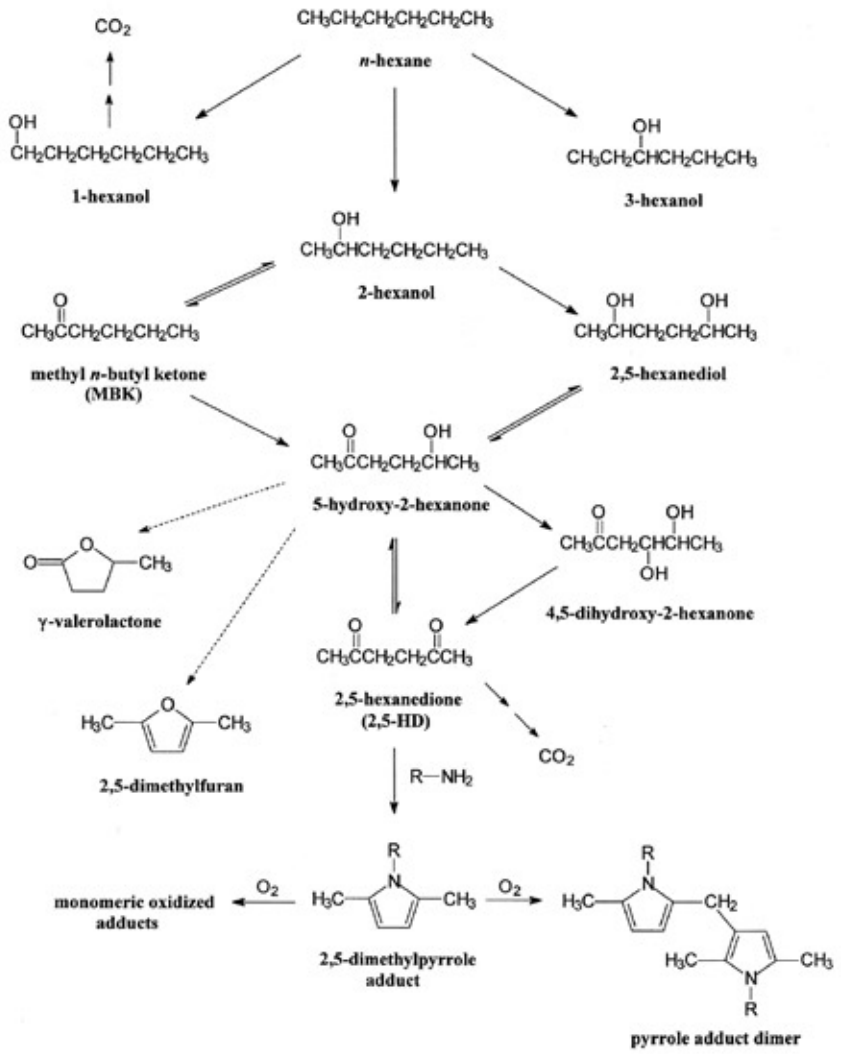

Figure 8. The metabolism of n-hexane. Methyl-n-butyl ketone and 2,5 hexanedione are particularly neurotoxic. Dashed lines indicate uncertain metabolic pathways. From Spencer *et al*., [31].

neurofilaments at the proximal paranodal region of the axon. Whether the involvement of the terminal boutons is always secondary to axonal damage is unclear – recent evidence suggests that the axon terminals may actually be the primary site of attack of a number of toxic agents (see later).

Myelinopathies

Myelinopathies are disorders of the myelin sheath and they are generally considered a subset of axonopathies. The demyelination of an axon may result from two distinct processes – directly by damage to the myelin sheath alone or indirectly via the Schwann cell. Two toxic agents which respectively illustrate the features of these two processes are hexachlorophene and diphtheria toxin. Hexachlorophene (Fig. 7) causes oedema between the myelin lamelli resulting in a spongiform appearance of the white matter of the CNS and conduction failure and neuromuscular paralysis peripherally. Schwann cell metabolism remains unaffected. In most cases axons are undamaged but axonal degeneration may occur in cases of severe poisoning. Diphtheria toxin produces a myelinopathy directly by interfering with myelin synthesis. The onset of myelinopathy is slow. In the case of hexachlorophene poisoning damage to the myelin occurs along the entire length of the axon. In the case of diphtheria, the loss of myelin may be

paranodal or involve the complete segment. In the latter, Schwann cell proliferation and remyelination is a conspicuous feature. Remyelination, when it occurs and whatever the cause of the initial demyelination, typically results in an unusually thin myelin sheath (Fig. 9) and numerous short internodes. In neither hexachlorophene poisoning nor diphtheria-induced demyelination is there a significant inflammatory response. The result of both conditions is slowed axonal conduction and in severe cases a total failure of conduction and severe neuromuscular weakness.

Ion channels and disorders of the peripheral nervous system

The normal functioning of the nervous system requires the regulation of the movement of charge across cellular membranes. This is accomplished by the controlled movement of ions – principally Na^+, K^+, Ca^{2+}, H^+, Cl^- - across excitable membranes via transmembrane ion channels. These ion channels may be passive, voltage gated or ligand gated or they may involve metabolically driven ion exchangers. Typical examples are the voltage activated channels for Na^+ and K^+ that control the generation of the action potential, the voltage gated Ca^{2+} channel associated with Ca^{2+} regulation in the nerve terminal of the motor axon, the ACh receptor of the post junctional membrane of the neuromuscular junction and the Na^+/ K^+ ATPase, an energy requiring pump that exchanges Na^+ for K^+ in a wide variety of cells. Ion channels are essentially composed of transmembrane proteins typically comprising two or more subunits that together form the ion pore and the regulatory machinery that determines the selectivity of the ion channel, the way the channels open, the speed of opening and closing, the duration of opening etc. Clearly, anything that disrupts the ion channels in terms of their function could have a significant impact on the nervous system.

Channelopathies as a class of diseases are uncommon and within the general class specific diseases are generally rare. They are caused by mutations in the genes responsible for the production of the channel proteins. Channelopathies are involved in clinical disease that affects the peripheral and central nervous systems, skeletal and cardiac muscle, the eye and kidney, and a variety of metabolic pathways. The diseases of the nervous system have been recently reviewed [19]. One disorder, affecting the voltage gated potassium channel, $K_v\alpha1.1$, located in the paranodal region of myelinated axons results in the delayed repolarisation of the membrane during action potential generation and the increased duration of excitability results in repetitive firing of axonal action potentials and neuromyotonia. The clinical features are muscle hypertrophy, muscle stiffness, cramps and weakness. An autosommal recessive disorder of mice (*med* and *jolting*) results from the impaired opening of neuronal Na^+ channels. It causes slow action potential conduction and functional denervation with axonal sprouting and muscle fibre atrophy (Fig. 10) as well as cerebellar ataxia.

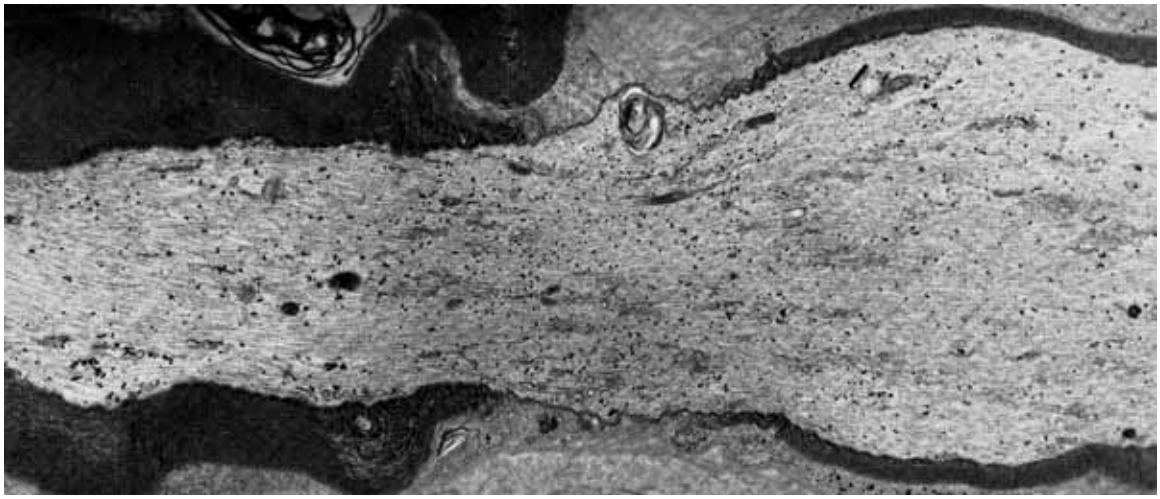

Figure 9. Paranodal region of a re-myelinated axon. The remyelinated internode (**right**) exhibits a very thin myelin sheath. From Schröeder [27].

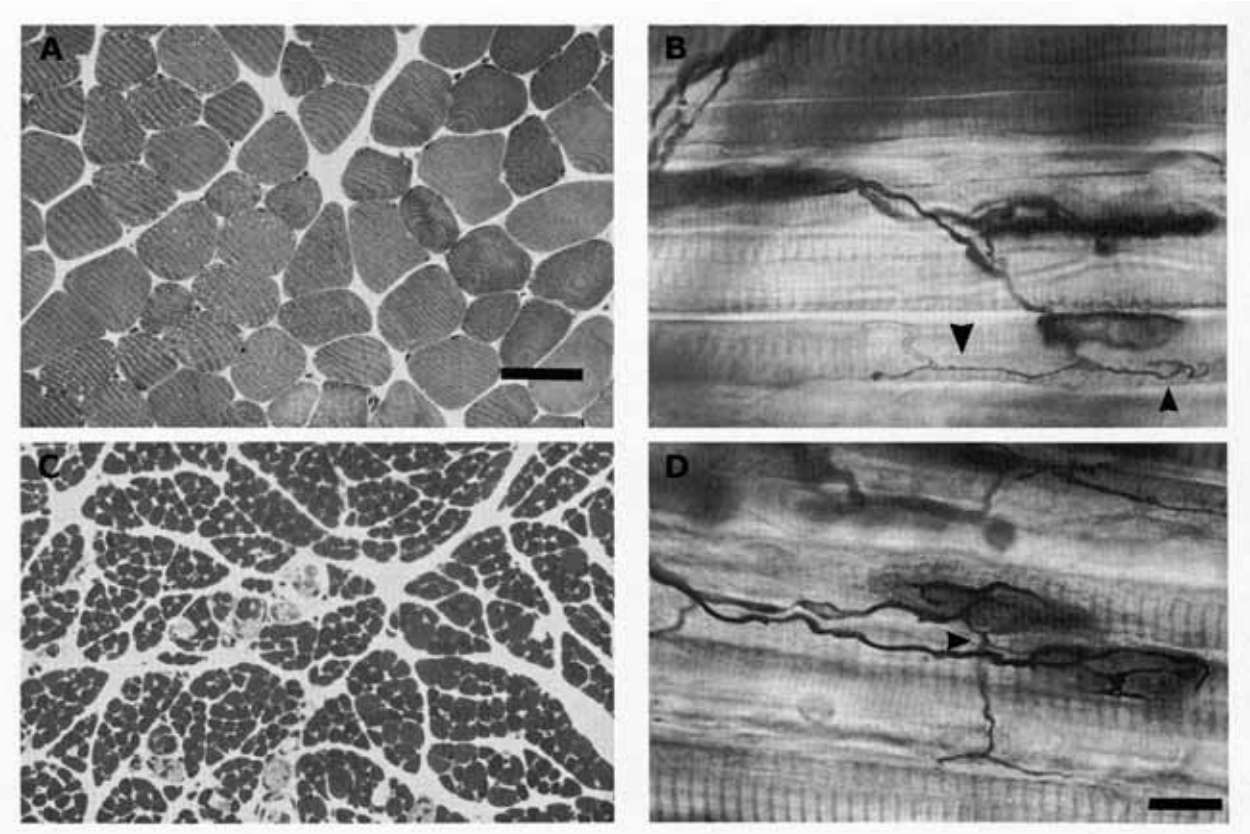

Figure 10. Transverse sections of biceps brachii muscles from phenotypically normal (**A**) and *med* mice (**C**) aged 21 days. Muscle fibers of *med* muscle (**C**) are severely atrophied. Bar = 50 μm. **B**, **D** Nerve terminal sprouting (**arrowheads**) at the motor end-plate of muscle fibers in the *latissimus dorsi* muscle of a *med* mouse. Bar = 20 μm. The *med* mutation results in reduced excitability of the voltage gated Na^+ channel and a functional denervation of the muscle fibres. Muscle fibre atrophy and nerve terminal sprouting are typical responses to functional as well as to structural denervation.

Many synthetic chemicals are neurotoxic as a result of their actions on specific ion channels. For example, DDT once widely used as an insecticide, activates voltage gated Na^+ channels and also slows Na^+ channel inactivation. This results in the prolongation of the action potential leading to hyperexcitability. DDT may also block Na^+/K^+ ATPase activity. Phenytoin, used in the management of seizures acts by stabilising the inactivated voltage gated Na^+ channel thus reducing excitability. Both compounds are very toxic in susceptible individuals.

Toxins of natural origin affecting ion channels are numerous. Toxins of plants include the pyrethroids, all of which act as insecticides by their actions on voltage gated Na^+ channels. The primary mode of action is the prolongation of Na^+ currents followed, at high doses, by the inhibition of both voltage gated Na^+ and K^+ channels. The prolongation of Na^+ current results in hyperexcitability and the generation of trains of action potentials following a single excitatory stimulus. Toxins of animal origin are much more common than toxins in plants as venomous animals have evolved toxins that target Na^+, K^+ and Ca^{2+} channels as part of their strategy for the capture of prey. The fish-eating cone-snails (genus *Conus*), for example, produce a variety of toxins that act by blocking voltage gated Na^+ channels and thus preventing action potential generation (members of this class of toxin are known as the μ-conotoxins). Another group of cone-snail toxins, the ω-conotoxins, block voltage gated Ca^{2+} channels and prevent transmitter release. Spiders and scorpions produce toxins acting on voltage gated Na^+ and K^+ channels. Some of these toxins are channel blockers, some activate the ion channels and some change the gating properties of the channels usually by preventing inactivation. Some sea-anemones are also producers of toxins directed towards voltage gated Na^+ and K^+ channels. In general terms, toxins that block voltage gated

K^+ channels or inhibit Na^+ channel inactivation prolong the duration of the action potential (Fig. 11) and render excitable cells hyperexcitable. Toxins that block voltage gated Na^+ channels block nerve conduction. Toxins that enable voltage gated Na^+ channels to open more easily may cause hyperexcitability, and may also reduce excitability as a result of the resulting depolarisation. Many toxins thought of as "animal toxins" are actually produced by micro organisms. For example the puffer fish, the blue ringed octopus and other animals known to be dangerous because they harbour tetrodotoxin do not themselves manufacture the toxin. Rather the toxin is produced by symbiotic micro organisms, probably species of *Vibrio.* Similarly, shell-fish responsible for paralytic shell fish poisoning are toxic because of the presence of gonyautoxins such as saxitoxin (Fig. 7) accumulated during feeding by the ingestion of marine dinoflagellates. Both tetrodotoxin and the gonyautoxins block voltage gated Na^+ channels. It is not possible to discuss in length the numerous toxins produced naturally that affect the nervous system, but the British Pharmacological Society produces guides covering the pharmacological actions of natural toxins [1] provides an excellent summary of the pharmacology of a number of the important natural toxins that affect the nervous system.

One defining feature of both inherited and acquired disorders of ion channels is that they are without obvious gross pathology. There may be pathology obvious only at the level of electron microscopy (see later) but the absence of obvious pathology is so common that it has been suggested that evidence of nerve conduction failure or hyper excitability in the absence of obvious structural change to the nervous system should be suggestive of a channelopathy.

Neuromuscular transmission

The best understood disease of the neuromuscular system is myasthenia gravis. In fact it is now clear that the term is usually used loosely to describe a large, heterogenous group of disorders that have as their origin a lowering of the safety factor of transmission (Table 1).

Many are autoimmune and others are the product of genetic abnormalities affecting the structural integrity of subunits of the acetylcholine receptor or other relevant components of the neuromuscular

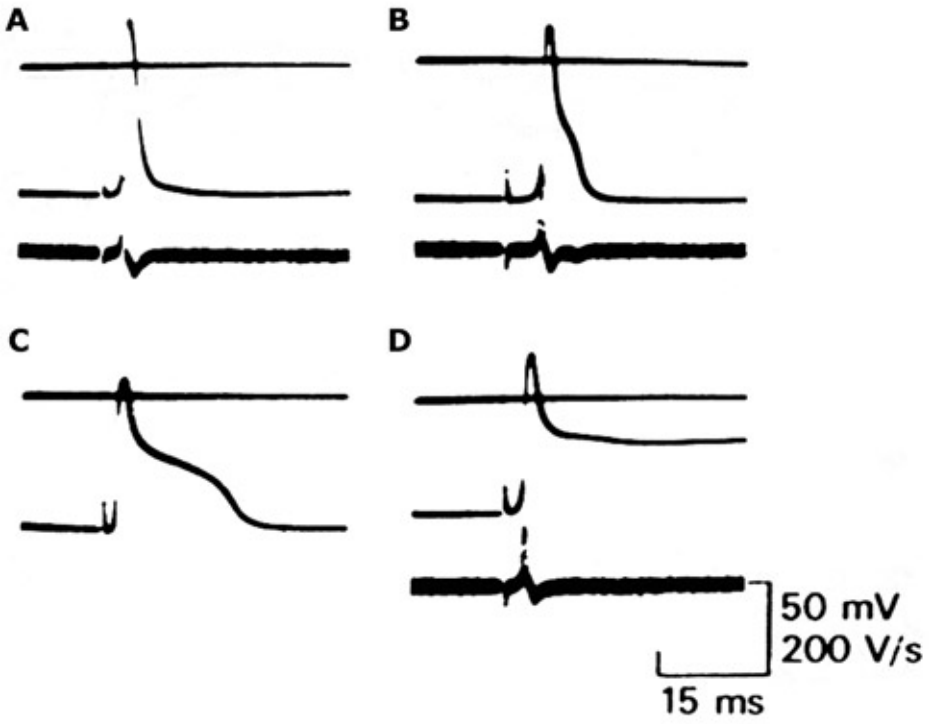

Figure 11. Action potential generation in rat diaphragm muscle fibres before (**A**) and after (**B**-**D**) exposure to the sea anemone toxin Atx-11. The toxin blocks inactivation of the voltage gated Na^+ channel, thus prolonging the duration of the action potential.

Table 1. Clinical and immunological characteristics of well-characterised autoantibody-mediated neurological disorders of the peripheral nervous system.

Disease	Pathology	Antigen	Diagnosis	Treatment
Myasthenia gravis	Loss of AChRs	AChRs	Anti-AChR	Responds to immunosuppression and thymectomy
	Loss of MuSK? Loss of AChRs?	MuSK	Anti-MuSK	Responds to immunosuppression
Lambert-Eaton syndrome	Loss of VGCCs	VGCC P/Q-type	Anti-VGCC	Plasma exchange, immunosuppression and intravenous immunoglobulin
Acquired neuromyotonia	Loss of VGKCs	VGKC on motor nerve	Anti-VGKC	Responds to anti-epileptic drugs and immunosuppression
Guillaine-Barré syndrome	Demyelination and/or axonal damage	GM1, GD1b and other glycolipids	Anti-GM1 in <50%	Plasma exchange and intravenous immunoglobulin
Miller-Fisher syndrome	Demyelination of ocular motor nerves?	GQ1b and other polysialylated glycolipids	Anti-GQ1b in >90%	Plasma exchange and intravenous immunoglobulin

AChrs: acetylcholine receptor; **VGCC**: voltage-gated calcium channel; **VGKC**: voltage-gated potassium channel (from Vincent and Martino [33]).

system. Classical myasthenia gravis is the most common disease of the neuromuscular junction. It is an autoimmune disease caused by antibodies that target the α-subunit of the ACh receptor. This results in a reduction in the safety factor of neuromuscular transmission by two mechanisms: the prevention of access to the receptor by ACh, and the complement-mediated lysis and accelerated internalisation of ACh receptors leading to a reduction of ACh receptor density. Maternal anti-ACh receptor antibodies can be transmitted via the placenta to bind to foetal ACh receptors and paralyse the foetus *in utero.* This neonatal form of myasthenia gravis is transient but it affects 10-20% of infants born to affected mothers. Other autoimmune diseases directed towards the postsynaptic components of the neuromuscular junction are those that target ACh receptors to cause the slow channel syndrome, those that enhance receptor desensitisation and those that attack MUSK, the muscle specific kinase that is essential for the agrin induced rapsyn-dependent clustering of ACh receptors at the neuromuscular junction. Another group of diseases results from the development of antibodies directed at voltage gated Ca^{2+} channels on the nerve terminal membrane, producing the myasthenia-like Lambert-Eaton syndrome in which transmitter release is impaired. Neuromyotonia (Isaac's Disease) is caused by auto-antibodies targeting voltage-gated K^{+} channels in paranodal regions of the motor axon [11]. Yet another group of diseases of the neuromuscular system, including principally Guillaine-Barré and Miller-Fisher syndromes, involve anti-ganglioside antibodies that bind to gangliosides in neuronal and glial cell membranes in nerve terminals and peri-synaptic Schwann cells to cause a depletion of synaptic vesicles and the complement mediated destruction of the motor nerve, demyelination, neurofilament disorganisation and axonal degeneration [35]. In view of the long held view that the nervous system is immunologically privileged [2] it is not clear why autoimmunity is such a pronounced feature of the pathology of the peripheral nervous system. It has been suggested that the lack of protection by a peripheral equivalent of the blood-brain barrier ensures that there are no restraints on access to circulating antibodies. This seems intuitively plausible but needs to be reconciled with the growing recognition that autoimmunity is the basis of a number of neurological disorders of the CNS, including seizures, memory loss, sleep disorders and multiple sclerosis. Finally, it should be noted that much autoimmune disease is paraneoplastic and has its origin in remote tumours. A recent detailed discussion of many aspects of autoimmunity in the nervous system may be found in [33].

Another major group of diseases of the neuromuscular junction is that comprising the congenital myasthenia syndromes. This is a heterogenous group of genetically determined diseases in which the safety factor of neuromuscular transmission is reduced. A number of mechanisms might be involved, and those identified to data include a mutation that reduces basal lamina ACh esterase activity resulting in the hyperexcitation of the post-synaptic membrane, localised muscle damage caused by the

excessive influx of Ca^{2+} and degradation of the synaptic folds; mutations affecting the size of quanta or the density of synaptic vesicles in the nerve terminal boutons; mutations that result in a change in ACh receptor kinetics; those that are associated with a reduced density of ACh receptors. This grouping is not exclusive. For example, reduced ACh receptor density may be associated with changed kinetics as well. The immature epsilon subunit of the ACh receptor is frequently the target of a mutation. This subunit is normally suppressed 12-16 weeks after birth but in affected subjects it would appear that the expression of the mature gamma subunit is accelerated. A full review of the complex molecular biology of the congenital myasthenic syndromes may be found in Engel and Sine [5].

The sensitivity of the synapse to autoimmune and genetic determinants of pathology is similarly expressed when the toxicology of the synapse is considered. Many of the most toxic natural products such as botulinum toxin, tetanospasmin and palytoxin target the nerve terminals. Botulinum toxin is the neurotoxic product of *Clostridium botulinum*. Seven distinct serotypes of the toxin are produced by different strains of *C.botulinum* (A, B, C, D, E, F and G). Toxin serotypes A and B are most often implicated in food borne poisoning. E and F are less often involved. Toxin serotype C is involved in wound botulism. Type A is by far the most toxic with an LD_{50} in mammals of <1 ng Kg^{-1}. Botulinum toxins are synthesised as a single molecule of 150 kD. As the toxin is secreted it is cleaved into two chains, heavy (H) and light (L) of 100 kD and 50 kD respectively, the two chains being attached by a single disulphide bridge. The toxins bind to a receptor (not yet characterised) on the nerve terminal plasma membrane via the H-chain and are internalised via receptor-mediated endocytosis. The internalized endosome is acidified by an endogenous proton pump. The acidification of the endosome results in the formation of a pore through which the L-chain passes. The disulphide bridge is reduced and the L-chain enters the cytosol. The L-chains are zinc-activated proteases that hydrolyse syntaxin and/or SNAP-25 or synaptobrevin, depending on the serotype of the toxin, thus preventing the docking of synaptic vesicles and transmitter release. Tetanospasmin, the neurotoxin produced by *Clostridium tetani* is structurally similar to the botulinum toxins, and its binding and internalisation into the nerve terminal is identical. In this case, however, the L-chain is transported back to the cell body and then moves transynaptically into the glycinergic inhibitory nerve terminals that provide an inhibitory input to the lower motor neurone. It cleaves synaptobrevin, thus preventing the docking of synaptic vesicles in the inhibitory neuron. The result is the unregulated excitation of the motor neuron and the well described spasms of tetanus. Other toxins target voltage gated Ca^{2+} channels in the nerve-terminal membrane to prevent transmitter release and neuromuscular paralysis (a typical example active on the voltage gated Ca^{2+} channels of the vertebrate nerve terminals is conotoxin ω-MVIIC from the cone-snail *Conus magus*). Palytoxin converts Na^{+}/K^{+} -ATPase into a non-specific ion channel, causing the depolarisation of excitable cells, muscle contraction, enhanced transmitter release and the depletion of synaptic vesicles and cardiac arythmias. It, too, is very toxic with an LD_{50} in mammals of approximately 0.5 μg kg^{-1}.

Among the most important natural toxins targeting the nerve terminal are the neurotoxic phospholipases A_2 that are common components of the venoms of many dangerous snakes, particularly members of the families Elapidae and Viperidae. These toxins form a sub-class of venom phospholipases A_2 many of which are purely digestive in function. The neurotoxic phospholipases A_2 appear to bind to the nerve terminals with a high degree of specificity. The hydrolytic activity of the toxins results in a depolarisation of the nerve terminal membrane and entry of Ca^{2+} via both the leaky plasma membrane and the opened voltage gated Ca^{2+} channels. This leads to an enhanced exocytosis, the depletion of synaptic vesicles (Fig. 4B) and eventually the degeneration of the terminal and the disruption of neurofilaments in the intramuscular components of the motor axons probably as the result of the activation of Ca^{2+} dependewnt proteases (Fig. 3D). The regeneration of the axon and its terminal results in extensive terminal sprouting (Fig. 3E, F). Bites by elapid snakes whose venoms are rich in these toxins cause a very severe neuromuscular paralysis that is relieved only by the regeneration of the damaged intra muscular axon and its terminals [23]. The precise details of binding and the mechanism of action of the toxins have not been unequivocally determined and remain the subject of considerable debate [21-23].

Several toxins cause an enhanced release of neurotransmitter as their primary mode of action. The most widely studied of these toxins is α-latrotoxin, a component of the venom of spiders of the genus *Latrodectus,* but it is a feature of the biology of numerous toxins in the venoms of spiders and scorpions. The toxin promotes the docking of synaptic vesicles, fusion and exocytosis, and the depletion of synaptic vesicles. The mechanism of action is complex and probably involves the interactions between the receptor for the toxin, synaptobrevin and the other toxins involved in exocytosis. The enhanced exocytosis is dramatic, miniature end-plate potential frequency, for example, increasing more than 100-fold. Degeneration of the nerve terminal may be a feature. Poisoning is easily reversed by treatment with anti-venom, a feature that is not the case with the presynaptically active phospholipases. Leptinotarsin, a toxin isolated from the haemolymph of the beetle *Leptinotarsa haldemani* also enhances exocytosis, but in this case, the enhanced exocytosis is caused by the opening of voltage gated Ca^{2+} channels.

Anticholinesterases that target acetylcholine esterase at the neuromuscular junction are relatively uncommon in the natural world. A small group of elapid snakes from Southern and Eastern Africa, the mambas (genus *Dendroaspis*) produce fasciculins, small polypeptides of 61 amino acid residues are active anticholinesterases. Anatoxon-a(s) (Fig. 7) is a natural anticholinesterase produced by some strains of the blue-green alga, *Anabaena flos-aquae*. This latter toxin is of some interest as it is a rare example of a naturally occurring organophosphate. Synthetic organophosphates are, however, very important compounds. They are widely used as plasticisers, expanders, antioxidants, flame retardants and insecticides. A small number are used as chemical weapons (i.e. the "nerve agents"). They have a common mode of action – the inhibition of acetylcholinesterase, thus causing hyperexcitability at all peripheral cholinergic synapses. In addition, a number of them (e.g. mipafox, paraoxon) can cross the blood-brain barrier, but it is not clear that penetration into the CNS causes anything more than a transient inhibition of acetylcholinesterase at central cholinergic synapses. The clinical effects of exposure to the organophosphates at the neuromuscular junction are neuromuscular weakness, fasciculation, respiratory distress and muscle pain, but with certain organophosphates these acute signs can progress to an intermediate syndrome 24-96 hours after exposure, characterised by profound neuromuscular weakness and ventilatory failure. The intermediate syndrome can last for 14-21 days. It resembles a peripheral neuropathy but no good pathological studies have been made. Muscle necrosis is a consistent feature, generally considered to reflect hyperexcitability of the muscle fibre and Ca^{2+} induced hypercontration and muscle degeneration at the junctional region. A more severe organophosphate-induced delayed polyneuropathy (OPIDN) may occur after exposure to a particular group of organophospahtes. The characteristics of the delayed polyneuropathy are a severe bilateral mixed sensory and motor polyneuropathy with flaccid paralysis. Recovery may be uneventful but residual problems are common. Nerve agents (e.g. soman and sarin) used a battlefield weapons are a particular class of organophosphate compound characterised by high toxicity and exposures at very high concentration and anecdotal evidence suggests that long term neurological and psychiatric problems are common. No good studies of affected subjects have been made, but neurological problems have been reported in animals [17].

There is remarkably little detailed evidence on the pathology and pathophysiology of the peripheral motor nervous system following exposures to organophosphates despite its clinical importance. There is some evidence of synaptic vesicle depletion and nerve terminal degeneration, and of Wallerian axonal degeneration in animals (see [14,17]). A good recent review of the toxicity of organophosphates may be found in Lotti [20].

The acetylcholine receptor is a major site for the activity of natural toxins. Of special importance and interest are the α-neurotoxins of snake venoms and cone-snails. The toxins bind, with varying degrees of affinity, to the α-subunits of the junctional ACh receptor and thus restrict the binding of acetylcholine to cause a potentially fatal neuromuscular paralysis. The neuromuscular weakness caused by the α-neurotoxins results in no obvious anatomical pathology.

The ACh receptor is rarely involved in neurotoxic incidents involving synthetic drugs and chemicals. The onset of neuromuscular disorder caused by such an agent would result in the acute onset of signs and

thus lead to an almost immediate ban on the use of such an agent.

Recovery and the return of function: an exercise in plasticity

It was pointed out earlier in this review that the nervous system is highly plastic, and this plasticity is a major feature of the response to toxin-induced damage and recovery. The primary distal response of the peripheral nervous system to acquired or inherited disorder is the dying back process that results in the withdrawal of the terminal from the neuromuscular junction. The biological processes underlying withdrawal are not well understood. The withdrawal of nerve terminals by motor neurons during the formation of a mature neuromuscular junction appears to be an active process whereby a neuron discards an inappropriate synapse and strengthens a more appropriate one. The process may involve the 'pinching-off' of a retracting nerve terminal by Schwann cell processes. The acute necrosis of nerve terminals does not appear to happen. Denervation by axonal section also results in the rapid loss of terminal structure and function [28]. Toxic damage caused, for example, by organophosphates, acrylamide, lead or the neurotoxic phospholipases causes withdrawal and 'pinching-off' but in addition terminal necrosis is a consistent (and probably dominant) feature [4,14,23,32]. Whether all of these processes involve common pathways and signalling systems for withdrawal and axonal degeneration is not at all clear – nor is the relationship between the destruction of the nerve terminal and the degeneration of the axon. There is a strong case to be made for the much closer integration of these studies. An excellent review addresses these issues [7] and abstracts of a recent Festschrift in honour of Professor C R Slater [25] provide relevant thinking on some of the issues relating to this topic.

The repair of neuromuscular junctions and the return of function also provide many examples of plasticity. Within a few hours after the separation of motor neuron from target muscle fibres there is an upregulation of the neuronal production and transport of signalling proteins and proteins of the axonal cytoskeleton, and the concentration of synapse specific proteins and synaptic vesicles in the growth cones of the regenerating axons [26].

The restoration of neuromuscular function begins with the close apposition of small nerve terminals and post synaptic structures, often from more than one motor neuron. Although the amount of transmitter released per nerve impulse is only a fraction of that released from a mature nerve terminal, the small diameter of the muscle fibre and its low membrane potential combine to ensure that the safety factor of neuromuscular transmission is sufficiently high to ensure effective transmission from very early stages of restoration (see for example [8]). Within 14 days or so, all of the induced changes in muscle properties mature, immature isoforms of the ACh receptor, and the voltage gated Na^+ channel are suppressed, and supernumery innervation is lost. Precisely the same sequence of events occurs with the contractile proteins and the proteins of the sarcoplasmic reticulum of the muscle fibres when they are damaged and repair. This topic is outside the scope of this review but has been reviewed recently [9].

FUTURE PROSPECTS

There is a growing interest in the role of environmental toxins in the development of neurodegenerative disease. Two thoughts dominate current thinking. The first is the possibility that exposure to neurotoxins might result in accelerated neuronal dysfunction leading, for example, to the early loss of motor neurons from the spinal cord and the early onset of neuromuscular weakness. In the central nervous system this might result in an accelerated decline in cognitive function or the spontaneous appearance of major degenerative disease such as Motor Neuron Disease or Parkinson's Disease. The second is that exposure in childhood might result in a loss of I.Q., behavioural problems and psychotic disorder in the long term. Thinking tends to be polarised, some believing the concept to be self-evident, others considering it so unlikely as to be improbable. Between the two opposing views the truth may lie: it is often not appreciated that only a small proportion of the population might be susceptible to most environmental neurotoxins and it is that subset of the population we need to identify. This might involve looking for those rare individuals in whom there is a genetic or immunologically based predisposition to neurological disease and

ask whether a disease process can be triggered by exposure to neurotoxic agents. Could, for example, autoimmune disease be triggered by exposure to toxic agents that cause a reversible neurodegeneration in the periphery, liberating potentially antigenic material into the circulation?

At the cellular level we need to ask whether the ultimate degenerative process, however cased, utilises the same intracellular signalling pathways. It is striking that the first response of the nerve terminal to such a wide range of toxic agents, including acrylamide, organophosphorous compounds, neurotoxic phospholipases and anti-ganglioside antibodies is the depletion of synaptic vesicles and nerve terminal degradation. Is there a common mechanism underlying this depletion? Is it possible that the selective and apparently transient damage caused by some of these agents could eventually lead to a peripheral neuropathy in susceptible individuals [18,23]? There is much still to do, and progress will be fastest if members of relevant disciplines and professions can work together.

REFERENCES

1. Alexander SPH, Mathie A, Peters JA (2006) Guide to receptors and channels. *Br. J. Pharmacol.* **147 (Suppl. 3)**, S1-S168.
2. Barker CF, Billingham RE (1977) Immunologically privileged sites. *Adv. Immunol.* **25,** 1-54.
3. Campbell MJ, McComas AJ, Petito F (1973) Physiological changes in ageing muscles. *J. Neurol. Neurosurg. Psychiatry* **36,** 174-182.
4. Duxson M J, Vrbova G (1985) Inhibition of acetylcholinesterase accelerates nerve terminal withdrawal at the developing rat neuromuscular junction. *J. Neurocytol.* **14,** 337-363.
5. Engel AG, Sine SM (2005) Current understanding of congenital myasthenic syndromes. *Curr. Opin. Pharmacol.* **5,** 308-321.
6. Freund H-J (1983) Motor unit and muscle activity in voluntary motor control. *Physiol. Rev.* **63,** 387-436.
7. Gillingwater TH, Ribchester RR (2003) The relationship of neuromuscular synapse elimination to synaptic degeneration and pathology: insights from Wlds and other mutant mice. *J. Neurocytol.* **32,** 863-881.
8. Grubb BD, Harris JB, Schofield IS (1991) Neuromuscular transmission at newly formed neuromuscular junctions in the regenerating soleus muscle of the rat. *J. Physiol.* **441,** 405-421.
9. Harris JB (2003) Myotoxic phospholipases A_2 and the regeneration of skeletal muscles. *Toxicon* **42,** 933-945.
10. Harris JB, Vater R, Wilson M, Cullen MJ (2003) Muscle fibre breakdown in venom-induced muscle degeneration. *J. Anat*. **202**, 363-372.
11. Hart IK, Maddison P, Newsom-Davis J, Vincent A, Mills KR (2002) Phenotypic variants of autoimmune peripheral nerve hyperexcitability. *Brain* **125,** 1887-1895.
12. Henneman E (1957) Relationship between size of neurons and their susceptibility to discharge. *Science* **26,** 1345-1347.
13. Henneman E, Somjen G, Carpenter DO (1965) Functional significance of cell size in spinal motor neurons. *J. Neurophysiol*. **28,** 560-580.
14. Hudson CS, Foster RE, Kahng MW (1985) Neuromuscular toxicity of pyridostigmine bromide in the diaphragm, extensor digitorum longus and soleus muscles of the rat. *Fundam. Appl. Toxicol.* **5,** S260-S269.
15. Huxley AF (1980) *Reflections on Muscle*. Liverpool University Press: Liverpool.
16. Katz B (1969) *The Release of Neural Transmitter Substances*. Liverpool University Press: Liverpool.
17. Kawabuchi M, Cintra WM, Deshpande SS, Albuquerque E X (1991) Morphological and electrophysiological study of distal motor nerve fiber degeneration and sprouting after irreversible cholinesterase inhibition. *Synapse* **8,** 218-228.
18. Kularatne SAM (2002) Common krait (*Bungarus caeruleus*) bite in Anuradhapura, Sri Lanka: a prospective clinical study. *Postgrad. Med. J.* **78,** 276-280.
19. Kullman D (2002) The neuronal channelopathies. *Brain* **125,** 1177-1195.
20. Lotti M (2000) Organophosphorus compounds In: *Experimental and Clinical Neurotoxicology*. 2nd edn. (Spencer PS, Schaumburg AS, Ludolph AC, eds). pp. 897-925. Oxford University Press: New York.
21. Montecucco C, Rossetto O (2000) How do presynaptic PLA_2 neurotoxins block nerve terminals? *Trends Biochem. Sci.* **25,** 266-270.
22. Prasarnpun S, Walsh J, Harris JB (2004) β-bungarotoxin-induced depletion of synaptic vesicles at the mammalian neuromuscular junction. *Neuropharmacology* **47,** 304-314.
23. Prasarnpun S, Walsh J, Awad SS, Harris JB (2005) Envenoming bites by kraits: the biological basis of treatment resistant neuromuscular paralyis. *Brain* **128,** 2987-2996.
24. Price J (2006) Stem cells in CNS repair. *Adv. Clin. Neurosci. Rehab*. **6,** 10-11.
25. Ribchester RR, McMahan VJ *et al.* (2006) Moving forward with neuromuscular junctions. *J. Anat.* **(in press)**.
26. Roos J, Kelly RB (2000) Preassembly and transport of nerve terminals: a new concept of axonal transport. *Nat. Neurosci.* **3,** 415-417.
27. Schröeder JM (2001) *Pathology of Peripheral Nerves*. Springer-Verlag: Heidelberg.

28. Slater CR (1966) Time course of failure of neuromuscular transmission after motor nerve section. *Nature* **209,** 305-306.
29. Slater CR, Harris JB (1994) The anatomy and physiology of the motor unit. In: *Disorders of Voluntary Muscle*. 6th edn. (Walton JN, Karpati G, Hilton-Jones D, eds). pp. 3-32. Churchill-Livingstone: Edinburgh.
30. Sofroniew MV, Howe CL, Mobley WC (2001) Nerve growth factor signalling, neuro-protection and neural repair. *Annu. Rev. Neurosci.* **24,** 1217-1281.
31. Spencer PS, Schaumburg HH, Ludolph AC (2000) *Exp. Clin.Neurotoxicol*. 2nd edn. Oxford University Press: New York.
32. Tsujihata M, Engel A G, Lambert E H (1974) Motor end-plate fine structure in acrylamide dying back neuropathy: a sequential morphometric study. *Neurology* **24**, 849-856.
33. Vincent A, Martino G (eds) (2002) *Autoantibodies in Neurological Diseases*. Springer-Verlag: Milan.
34. Whalen RG, Harris JB , Butler-Browne GS , Sesodia S (1990) Expression of myosin isoforms during notexin-induced regeneration of rat soleus muscles. *Dev. Biol.* **141**, 24-40.
35. Willison JHJ (2005) The immunobiology of Guillain-Barre syndromes. *J. Peripher. Nerve Syst.* **10,** 94-112.

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