Threshold Electrotonus and Ion Channel Dysfunction in Motor Neurone Disease

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Abstract

Threshold electrotonus is a new neurophysiological technique, which records excitability changes in axons induced by subthreshold currents and detects abnormalities in internodal, as well as nodal, nerve membrane. The technique provides a unique means of studying the presumed membrane instability responsible for the generation of fasciculations in motor neurone disease. An initial clinical study using the technique, revealed that eleven patients with motor neurone disease exhibited consistently abnormal changes in excitability produced by 100 ms polarising currents, compared with fifteen normal and nineteen neurological controls. The abnormality was most pronounced 10 – 20 ms after the onset of the current, suggesting reduced activity of fast and slow potassium channels. The current study was undertaken to assess the reproducibility of these findings and the value of threshold electrotonus as a diagnostic and prognostic tool in motor neurone disease. The study, also, aimed to appraise the effect of riluzole, which has an action on sodium channels, and membrane stabilising medication. Seventy patients with motor neurone disease were studied and were compared to thirty-five normal and sixty-four neurological controls. Thirty-seven of the motor neurone disease patients were followed up every two months for up to two years. The results of the recordings are presented and reveal that although threshold electrotonus is a very specific test for motor neurone disease, it is not as sensitive as was previously expected. Additional data are also presented on the effect of temperature, the use of single unit recordings and the use of the technique in patients with multiple sclerosis, who were found to exhibit a novel abnormality which correlated with disease activity.
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1. INTRODUCTION

1.1 THRESHOLD ELECTROTONUS - INTRODUCTION

'Threshold electrotonus' is a new adaptation of a very old electrophysiological technique, which was devised by Bostock and Baker (1) to identify the role of potassium channels in axons. The technique is based upon the indirect measurement of electrotonus by studying the processes of accommodation or changes in excitability in nerve. The method, which will be discussed in more detail in later chapters, tests excitability in the axon, with a brief test shock applied at various times during and after a rectangular, subthreshold, conditioning current pulse. The subthreshold conditioning currents induce electrotonic changes in potential in the axon. These electrotonic changes in membrane potential or 'electrotonus' represent the reaction of the membrane to applied currents that do not induce excitation.

As early as 1859 Pfluger (2) was studying 'electrotonus' in frog nerve. However, his definition of electrotonus varied somewhat from currently accepted definitions, as his experiments involved the measurement of changes in muscle twitch to a submaximal stimulus. In 1931 Erlanger and Blair (3), made measurements corresponding more accurately to threshold electrotonus. They recorded changes in threshold current to a subthreshold conditioning current in frog nerve, in order to study what they eventually termed 'cathodal depression' (4) and is now known as subthreshold accommodation. Subsequently, Lorenté de Nó in 1947 (5) concurred with Rosenblueth's previous observation (6) that a close parallel exists between the accommodative changes in excitability recorded from nerve and the electrotonus. Lundberg (7) then recorded a slow relaxation of a potential change induced by hyperpolarising
currents from frog spinal roots and desheathed nerves, which was similar to Lorente de Nó's (5) description of 'nerve reaction'. Following these experiments, further studies of the full components of electrotonus disappeared from the literature. The classical models of myelinated nerve (8;9) and even Barrett and Barrett's (10) revised cable model accounted for only one of the slow components of electrotonus (see section 1.3).

With the advent of their interest in the study of accommodation in nerve as a means of investigating the role of potassium channels, Baker and Bostock (11) developed the technique of threshold electrotonus. They confirmed the previous descriptions of the strong relationship between the threshold measurements that were recorded and the electrotonic potentials of the nerve. The 'threshold', in this context, is defined, as the strength of a current pulse required to excite a single nerve fibre, or to excite a specified fraction of a maximal compound response. The term 'threshold electrotonus' was thus coined (1) and refers to the changes in threshold corresponding to electrotonus, particularly changes in threshold caused by long duration (50 - 500 ms) current pulses measured with a short duration (1 ms) test pulse.

The technique of threshold electrotonus differs from early methods of studying excitability change or accommodation of nerve in three main ways. First, the original investigators used a constant stimulus to monitor excitability change. With the aid of modern technology, it is now possible to 'track' the threshold by computer feedback and therefore maintain a constant response by altering the stimulus intensity. Secondly, by expressing the polarising currents and excitability changes as dimensionless parameters, a method has been created whereby comparisons can be made between different species and between different nerves in the same species, irrespective of the total amounts of current applied. The magnitude of the polarising currents are expressed as a fraction of the threshold current for a 1 ms test pulse and the
excitability changes as a percentage reduction in threshold. Lastly, the technique has provided a non-invasive and well-tolerated means of recording human nerves in vivo, which accesses information about the internodal, as well as nodal, regions of the nerve.

Prior to further discussion about the technique of threshold electrotonus, it is necessary to consider the concepts of accommodation and electrotonus in more detail.

1.2 ACCOMMODATION

The term 'accommodation' when applied to nerve has acquired two definitions. The first was originally developed by Nernst (12) in 1908 to describe the process by which a nerve would no longer be excited by a stimulating current, if the current were increased sufficiently slowly. This definition corresponds to Blair and Erlanger's (4) description of 'cathodal depression' but is currently termed 'subthreshold accommodation'. The second definition, however, refers to the process whereby a sustained suprathreshold current pulse causes a limitation in discharge of the nerve and has additionally been termed 'adaptation'. Further references to 'accommodation' shall be used to describe any reduction in excitability caused by prolonged stimulation whether by subthreshold or suprathreshold currents and the alternative terms shall be used to distinguish the two aspects of accommodation.

In a biological context, axons are constantly subjected to naturally occurring, slowly changing stimuli. Therefore, the processes of subthreshold accommodation and adaptation provide a means whereby a control is placed on how frequently the axon discharges on excitation.

Accommodation was one of the few properties of nerve that was measurable prior to the advent of more modern techniques such as studies on isolated fibre preparations or voltage clamp experiments, and
there is an extensive early literature on the subject. In 1848, Du Bois-Reymond (13) pointed out that it is the change in current intensity, rather than the current density at any given moment, that determines the effectiveness of a stimulus to the nerve. Moreover, he remarked that the more rapidly the current changes the greater the response. Similarly, Lucas (14) found that in order to excite frog nerve, a minimal gradient or 'critical slope' was required for a linear current ramp. The ratio between the rheobase and this gradient gives a time constant, which provides a measure of accommodation, such that the shorter the time constant, the 'better' the accommodation. Throughout the 1930's Blair and Erlanger (3;4;15) carried out a series of experiments and as mentioned previously, discovered that the increase in excitability caused by sustained depolarising currents is followed by a depression in excitability which they termed 'cathodal depression'. They additionally described poorly adapting fibres as exhibiting 'repetitiousness' (16). Kugelberg (17) described the impression that 'excitability' of human nerve gained by an examining physician was closely related to its accommodation.

The only explicit mathematical theory of accommodation that exists was proposed by Hill (18) in 1936. Unfortunately, he made two simplifying assumptions that proved to be incorrect for normal nerve fibres, although they describe quite well the behaviour of some depolarised fibres (19). Where the theory fell short, is that Hill firstly assumed that accommodation was due to a change in voltage threshold, not membrane potential. Secondly, he thought that accommodation to a maintained current was eventually complete, so that the threshold tends to return to the baseline, regardless of how it has been disturbed. Even at the time these limitations were partially realised. Solandt (20) pointed out that it was necessary to soak the nerve preparations in potassium-rich solutions 'to remove the excitability changes due to electrotonus'
and it subsequently became apparent (19) that Hill's theory only applies to axons that are depolarised.

The clinical importance of studying accommodation is based on a greater understanding of the failure of the process, which is fundamental to the generation of the ectopic discharges responsible for paraesthesiae and fasciculations. As will be described later, an initial clinical study recording threshold electrotonus in patients with a variety of neurological conditions (21), indicated that abnormal accommodation, possibly caused by dysfunctional potassium channels in the axon, may be at least partly responsible for the neurodegenerative process in motor neurone disease (MND). The development of the technique of threshold electrotonus has provided a non-invasive means of recording subthreshold excitability changes that constitute accommodation and furthermore provides information about membrane properties and ion channels in vivo.

1.3 ELECTROTONUS

Electrotonus represents the responses of the nerve membrane to currents that do not induce excitation. These can be subthreshold depolarising or hyperpolarising currents. By varying the amplitudes of the polarising currents in the depolarising and hyperpolarising directions, characteristic traces emerge (Fig 1.1). Lorenté de Nó (5) first noted that electrotonus in myelinated nerves is characterised by a fast component, with a rise time of <1 ms, followed by slow potential changes, which are more sensitive to membrane potential.

A representative example of electrotonic responses is shown in Figure 1.1 (see Baker et al 1987) (11).
Figure 1.1: Typical Electrotonus Waveforms

Typical electrotonic potentials (V) evoked by constant current pulses (I). Fast (F) and slow (S1-S3) components are indicated (See text for full description).

[Figure taken from Baker, Bostock, Grafe and Martius, Function and distribution of three types of rectifying channel in rat spinal root myelinated axons. J Physiol 1987;383:45 – 67].
Figure 1.2: Simplified Equivalent Circuit of Node and Internode of Myelinated Axon Showing Principal Electrical Components.

A computer model based on this circuit can simulate threshold electrotonus. Each ion type is represented by a cell. The electrogenic ion pumps are represented as current sources ($I_{\text{pump}}$). Currents generated by the nodal membrane (main block on left) or applied from outside, access the internodal axon membrane (main block on right) via the capacitance of the myelin sheath ($C_m$) and the internodal leak resistance ($R_i$). As a result of its high electrical capacitance ($C_i$), changes in the membrane potential of the internodal axon are about three times slower than the nodal capacitance ($C_n$). The nodal channels illustrated are: **Na**, sodium channels responsible for the action potential; **Nap**, persistent sodium channels, active in subthreshold potential range, they lower the rheobase, prolong the strength-duration time constant and facilitate repetitive firing; **Kf**, fast potassium channels, present but of little importance at the nodes; **Ks**, slow potassium channels, responsible for the S2 component of threshold electrotonus, reduce excitability both by hyperpolarisation and by increasing nodal conductance. The internodal ion channels illustrated are: **Na**, internodal sodium channels probably outnumber nodal channels, not normally activated but may contribute to pathological discharges and assist conduction in demyelination; **Kf**, fast potassium channels, present in high concentrations at the internodal portion of the axon, limit depolarising electrotonus and together with the slow potassium channels, **Ks**, contribute to the maintenance of the resting potential; **IR**, inward rectifier channels, activated by hyperpolarisation, are responsible for the S3 component of electrotonus; **Lk**, leak or non-voltage-dependent channels, contribute little to electrotonus.

As a result of the sweep duration, the fast component (F) appears virtually instantaneous and is almost symmetrical in the depolarising (upwards) and hyperpolarising (downward) directions (Fig. 1.1). There are subsequently three slow components that can be distinguished. Firstly, S1 which is a slow potential change in the same direction as the fast electrotonus, seen in response to small current pulses in either direction. It can be exaggerated by hyperpolarisation. The second component is S2, which is an outwardly rectifying conductance seen in response to strong depolarising currents and produces a delayed sag in the trace. The third slow component is S3 which is an inwardly rectifying conductance in response to strong hyperpolarising currents and produces a late upwards recovery of the hyperpolarising trace. This current is more significant in sensory rather than motor nerves.

Finally, an intermediate outwardly rectifying component of electrotonus can be distinguished (R1). This has a time course between the fast and slow components and produces a downward notch in traces produced by strong depolarising currents. However, R1 is only found at levels of stimulation above threshold and therefore higher than would be produced by the technique of threshold electrotonus. The presence of R1 became apparent in these experiments (11), as the rat nerve was treated initially with tetrodotoxin (TTX), which prevented the generation of action potentials.

The fast component (F) represents the cable response determined by the capacitance and resistance of the node and the capacitance of the myelin sheath (22). However, a simplified model can be constructed (Fig 1.2) in which the amplitude of the fast response depends on the nodal resistance and the resistance of the path to the internodal axolemma (11). In this model, changes in the fast electrotonus normally represent changes in nodal resistance. The S1 component of electrotonus represents the passive spread of charge to the internodal
axolemma. Barrett and Barrett's (10) model predicts the exponential behaviour of the responses to depolarisation and hyperpolarisation. The asymmetry that exists represents the function of the voltage-dependent conductances and is better illustrated by Bostock's model (Fig. 1.2).

Many authors (23-25) have proposed the hypothesis that the nerve membrane contains different classes of potassium channel. Schwarz and Vogel (23), for instance, showed that stationary inactivation of $I_k$ remains incomplete even with large depolarising pulses and moreover, inactivation of $I_k$ develops in two phases - fast and slow inactivation.

However there do appear to be marked differences between the properties of potassium channels in invertebrates, especially frog nerve, and those in mammals. Hille (26) showed that tetraethylammonium chloride (TEA), inhibited a fast potassium current in frog nerve. Dubois (27), further delineated three potassium conductances in frog node of Ranvier. He named them $K_s$, $K_{t1}$ and $K_{t2}$ to represent a slow and two fast potassium conductances. The fast conductances were found to be sensitive to 4-aminopyridine (4AP) which was known to block voltage-dependent potassium current in invertebrate nerves (28). However, the slow conductance was unaffected by 4AP. Moreover, both the fast and slow conductances were blocked by tetraethylammonium chloride (TEA).

One can compare invertebrate and mammalian potassium channels by their current properties or the sensitivity to various blocking agents. Brismar (29) and Brismar and Schwarz (30) described potassium currents, with properties to match Hille's (26) frog node 4AP - sensitive conductance, in voltage-clamped rat nodes. These proved to be sensitive to 4AP but not to TEA, indicating a primary difference between frog and rat nerve. Bowe et al (31), studying mammalian fibres, found that immature dorsal root fibres treated with 4AP fired bursts of action
potentials in response to a single brief stimulus, and thus showed the functional importance of these channels. A slowly activating potassium current was subsequently described by Goren et al (32) in mammalian nodes under voltage clamp. This slow TEA-sensitive potassium conductance, which is not sensitive to 4AP, is important for accommodation, especially in motor fibres. Its most important effect is probably to limit ectopic firing secondary to maintained depolarisation such as minor trauma and ischaemia, which might otherwise be exaggerated and prolonged intolerably, despite the accommodating effects of sodium inactivation.

Frog nerve therefore contains three known potassium conductances, two that are fast and sensitive to 4AP and TEA, and one that is slow that is only sensitive to TEA. In rat nerve, however the fast potassium conductance is only sensitive to 4AP and the slow conductance to TEA. Scholz et al (33), provided the first direct electrophysiological information on the molecular basis of human nerve excitability and revealed the presence of two fast conductances which they termed I channels and F channels, which appear to correspond to the two fast conductances found in frog nerve by Dubois (27). Following Dubois's terminology, these were subsequently named Kf1 and Kf2 (34). However, as with rat nerve, these fast conductances in human nerve are only sensitive to 4AP and are relatively insensitive to TEA. The presence of a slow TEA-sensitive potassium conductance, subsequently named Ks after Dubois (27), was also confirmed (34). The differences found between the electrical properties of rat and human nerve are likely not to be due to the presence of fundamentally different channel types, as are found between frog and mammalian nerves, but due to differences in channel density or distribution (33).

Lorento de No (5) described the phenomenon of 'nerve reaction' as a slow relaxation of the potential change induced by hyperpolarising currents, when he recorded from whole frog nerve. Lundberg (35)
recorded similar potentials from frog spinal root and desheathed nerves and thus showed that this 'nerve reaction' was not a function of the nerve sheath. Isolated single fibres then became the most common preparation for experiments and the term 'nerve reaction' disappeared, to be replaced with the terms 'anomalous rectification' and 'inward rectification' and more recently superseded by the terms \( I_H \) or \( I_Q \) after more precise biophysical descriptions of the current (36). These describe conductances activated by hyperpolarisation in muscle fibres and nerves. Two types of inward rectifier have been distinguished (37). The first is relatively potassium-specific and is blocked by barium (\( \text{Ba}^{2+} \)) and caesium (\( \text{Cs}^+ \)) ions. It is found in egg cells, muscle fibres and olfactory cortex neurones. The second has an appreciable sodium conductance and is \( \text{Cs}^+ \)-sensitive, but \( \text{Ba}^{2+} \)-resistant. It is found in Purkinje fibres and many neurones, including dorsal root ganglion and motor neurones. The most likely function of inward rectification in myelinated nerve is to limit the electrogenic hyperpolarisation and consequent reduction in excitability (11). This may be of critical importance in damaged nerve and in regions of reduced safety factor, such as branch points and nerve terminals.

Baker et al (11) found that in rat spinal roots, the use of 4-AP, TEA and caesium ions (\( \text{CS}^+ \)), could differentiate three different conductance systems and thereby differentiate three voltage-dependent components of electrotonus.

When studying rat spinal roots treated with TTX and TEA, 4AP was shown to have two separate effects, both distinct from the action of TEA. The first was to affect the S1 component of electrotonus (Fig 1.1) which occurs with subthreshold depolarising currents producing an enhancement of the early response to depolarisation or causing a more rounded appearance of the flat top of the depolarising response. The measurement of the fast and slow components showed that the increase in depolarising electrotonus occurred mainly after the first 0.5
ms and that the amplitude of the fast component at the end of the pulse was only slightly altered by 4AP. This indicates that the substantial increase in slow electrotonus could not be explained by block of nodal conductance. The second effect of 4AP was to abolish the R1 component (Fig. 1.1) found when the strongest depolarising currents were used. On comparison of electrotonic waveforms before and after the application of 4AP, it becomes evident that R1 can be ascribed to a 4AP-sensitive delayed rectification that becomes faster with increasing depolarisation. R1 is also associated with a reduction in fast electrotonus and therefore increased nodal conductance. 4AP does not affect the response to hyperpolarisation. The action of 4AP on the internodal axon is probably a combination of the blockage of some channels open at rest and of others that open on depolarisation.

TEA on the other hand blocks the slow outwardly rectifying S2 sag in the electrotonus waveform in response to strong depolarising currents. S2, like the faster R1, produces a reduction in fast electrotonus, and similarly, the responses to hyperpolarisation are not affected by TEA. Nodal TEA-sensitive channels take at least 0.5 ms to be activated. Moreover, since TEA-sensitive rectification is recorded after 50ms, internodal channels must also exist. There is probably a greater density of channels in the internodal region and may thus require less depolarisation to generate a detectable conductance.

Cs+ rapidly and reversibly reduces the inwardly rectifying component of electrotonus (S3) that is found in response to strong hyperpolarisation. Inward rectification or CS+ does not affect the amplitude of the fast electrotonus. Thus inward rectification is likely to be a property of the internodes alone. In these experiments on rat spinal nerves, Ba^{2+} did not imitate the action of Cs^+.
1.4 BACKGROUND OF THE STUDY

The clinical applications of the study of electrotonus and accommodation was developed by Bostock et al (21) and provided the basis for this study.

As described above, electrotonus waveforms provide specific information about the individual properties of various channels found in the nerve membrane. Dysfunction of these channels would be likely to alter the excitability of the nerve membrane and may thus affect the normal pattern of firing, possibly underlying the generation of fasciculations and paraesthesiae.

Fasciculations are a characteristic feature of motor neurone disease (MND, amyotrophic lateral sclerosis or ALS) and represent spontaneous motor unit discharges (38;39). Fasciculations are also found in many lower motor neurone conditions and in some normal subjects ('benign fasciculations'), however they can usually be differentiated electromyographically from those found in MND by certain criteria. The fasciculations found in MND tend to occur at a slow rate (0.3 Hz) and at irregular intervals (40;41). Whereas, those associated with root or peripheral nerve disorders, or those found in normal subjects occur at a more rapid rate (about 1 Hz) and more regularly. The clinical features and the pattern of the motor unit potential can also help to differentiate the various groups. In normal subjects fasciculation potentials consist of normal motor unit potentials and, in addition, are not accompanied by atrophy and weakness or by EMG features of chronic partial denervation. The fasciculations of MND are often clinically larger and more obvious than those found in root or peripheral nerve disorders and on EMG the fasciculation potentials are widespread, not being confined to a particular root or nerve territory. They are also more polyphasic indicating more marked enlargement of the remaining motor units in partially denervated muscles as a result of collateral sprouts developing.
Fasciculations in MND were historically thought to arise proximally from the diseased soma of the anterior horn cell (42) but are now known to be multifocal in origin. It has been shown that fasciculations may persist after more proximal nerve block (43) and that 80% of fasciculations are not abolished by collision techniques (44), thus indicating a more distal origin. Other studies have shown that fasciculations can arise proximally (less commonly), distally or at multiple sites within the motor unit (45-47). Therefore, it is likely that in MND there is a widespread disturbance of axonal membrane excitability found throughout the motor unit.

The technique of threshold electrotonus is unique in its ability to provide information about the activation of ion channels under the myelin sheath. It was therefore felt to be an ideal test for the proposed disturbance in excitability found in MND, since the majority of axonal ion channels contributing to the resting potential and to membrane stability are internodal.

1.4.1 Motor Neurone Disease

Prior to the description of the initial clinical study using the technique of threshold electrotonus, it is necessary to expand on the clinical and pathophysiological features, in addition to the aetiological theories of MND, as the disease is the central focus of this study.

1.4.1.1 Definitions and Terminology

MND is a degenerative disease of the upper and lower motor neurones with progressive weakness of bulbar, limb, thoracic and abdominal muscles. The disease can present with weakness in the bulbar region or any of the spinal segments. There is relative sparing of oculomotor and sphincter function. The sensory system is unaffected clinically and with routine neurophysiological tests, although some patients occasionally complain of sensory symptoms.
The disease is characterised by muscular weakness and wasting, with fasciculations (which are often prominent and may precede other symptoms by years) and by spasticity, hyperreflexia and extensor plantar responses. Bulbar involvement is common, causing dysarthria and dysphagia. Death usually follows within five years from presentation, often from ventilatory failure.

The term ‘motor neurone disease’, introduced by Brain (48), is used generically in the United Kingdom to included the complete range of the presentations of the disease. Whereas the term introduced by Charcot (49), ‘amyotrophic lateral sclerosis’ (ALS), is used in a similar capacity in the United States and much of Europe. Further use of the terms ‘MND’ and ‘ALS’ will follow the British classification.

According to British terminology, ‘MND’ covers four variations of presentation of the disease: A) ‘Amyotrophic lateral sclerosis’ (ALS) - with a combination of upper and lower motor neurone signs in the spinal segments +/- the bulbar region; B) ‘Progressive muscular atrophy (PMA) - with only lower motor neurone involvement; C) Progressive bulbar palsy - with lower motor neurone bulbar involvement and D) Pseudobulbar palsy - with upper motor neurone bulbar involvement. A further sub-group is considered within this study, namely ‘primary lateral sclerosis’ (PLS), in which progressive upper motor neurone degeneration is not accompanied by anterior horn cell degeneration. The exact relationship of PLS to the other forms of MND remains uncertain.

While most cases of MND are sporadic, about 5 - 10% of cases are familial, usually with autosomal dominant transmission. As with sporadic MND there is significant phenotypic variation with familial MND. Mutations of the gene encoding for Cu,Zn superoxide dismutase on chromosome 21, have been found in some families with MND (50),
however the majority do not show this mutation and thus the search for other MND genes continues.

1.4.1.2 Epidemiology

The incidence of MND is about 2 - 3 per 100,000, with a prevalence of 4 - 7 per 100,000, in most parts of the world. A much higher incidence is found in the Western Pacific, however the numbers of new cases are falling in this region (51).

Men are more commonly affected than women with a ratio of 1.5: 1. The incidence of the disease increases with age, with a peak between 60 and 70 years of age (52). The mean survival in patients with predominantly spinal forms of the disease i.e. ALS, was found in one study, to be 3.3 years, whereas those with bulbar symptoms survived 2.2 years (53). Other studies have found the median survival for the whole group to be about 3.5 years from the onset of symptoms (54;55). Risk factors for the disease are increased age, male sex, mechanical injury and having lived for many years in certain parts of the Western Pacific.

1.4.1.3 Clinical Diagnostic Criteria

With a view to developing workable, internationally - acceptable diagnostic criteria for motor neurone disease, The World Federation of Neurology held a consensus conference in El Escorial in Spain in 1990 (56). The resultant diagnostic criteria are listed in Table 1.1 and have provided the basis for selecting patients for this study.

In addition to the clinical criteria described, other features and results that support a diagnosis of motor neurone disease include: fasciculations in one or more regions; normal motor and sensory nerve conduction with no conduction block; signs of partial chronic denervation on electromyographic examinations and minimal abnormalities on head or spinal cord magnetic resonance imaging (MRI).
1.4.1.4 Electrophysiological Diagnostic Criteria

Electrophysiology is an extremely important investigation in the assessment of patients with motor neurone disease. The initial criteria were outlined by Lambert in 1969 (38) and still provide the gold standard (see Table 1.2), however Behnia and Kelly (57) more recently made several additional points that outline some of the limitations of EMG in the diagnosis of MND (Table 1.2).

The diagnosis of motor neurone disease / amyotrophic lateral sclerosis requires the presence of:
- Signs of lower motor neurone (LMN) degeneration by clinical, electrophysiological and neuropathological examination.
- Signs of upper motor neurone (UMN) degeneration by clinical examination.
- Progressive spread of signs within a region or to other regions.

Clinical diagnostic criteria:
- **Definite ALS**: UMN, as well as LMN, signs in the bulbar region and at least two of the other spinal regions (brachial, thorax, trunk, and crural), or UMN and LMN signs in three spinal regions.
- **Probable ALS**: UMN and LMN signs in at least two regions, with UMN signs rostral to LMN.
- **Possible ALS**: UMN and LMN signs in one region or UMN signs alone are present in two or more regions or LMN signs are rostral to UMN signs.
- **Suspected ALS**: LMN signs in two or more regions.

Inconsistent clinical features:
- Sensory dysfunction.
- Sphincter abnormalities
- Autonomic nervous system dysfunction.
- Anterior visual pathway abnormalities.
- Movement disorder

Table 1.1. Clinical Diagnostic Criteria for Motor Neurone Disease / Amyotrophic Lateral Sclerosis, based on the El Escorial Criteria.

The term 'ALS' in this table refers to the European definition, and therefore pertains to all subgroups of motor neurone disease.
Lambert's Diagnostic EMG Criteria for MND (38)

1) Fibrillations and fasciculations in muscles of the lower and upper extremities, or in the extremities and the head.
2) Reduction in number and increase in amplitude and duration of motor unit action potentials.
3) Normal electrical excitability of remaining fibres of motor nerves and motor fibre conduction velocity within the normal range in nerves of relatively unaffected muscles and not less than 70% of the average normal value according to age in nerves of more severely affected muscles.
4) Normal excitability and conduction velocity of sensory nerve fibres, even in severely affected extremities

Limitations Suggested by Behnia and Kelly (57)

1) Conduction studies may be unreliable in motor nerves with markedly low compound muscle action potential (CMAP) amplitudes.
2) Sensory nerve action potential (SNAP) amplitudes may be abnormal in a small percentage of otherwise typical MND patients, though better controls for elderly subjects are required.
3) EMG may not show widespread active denervation early in the disease.
4) Some patients may have a mild polyneuropathy.

Table 1.2. EMG Diagnostic Criteria for Motor Neurone Disease.

Based on Lambert's (38) original criteria with additional limitations suggested by Behnia and Kelly (57).

The EMG abnormalities found in MND are not, however, specific to the disease. Whereas the EMG can distinguish MND from myopathies and diseases affecting only the upper motor neurone, very similar abnormalities are also found in other anterior horn cell diseases such as the spinal muscular atrophies and poliomyelitis or focally in localised spinal cord lesions such as syringomyelia and spondylotic myelopathy. In these situations it is especially important to take into account the history, examination and other investigations, such as MRI, to provide the most likely diagnosis.
However, situations arise with certain disorders, in particular multifocal motor neuropathies, when the clinical picture is indistinguishable from MND and nerve conduction studies are of paramount importance in clarifying the diagnosis for these potentially treatable conditions (58). In the form of multifocal motor neuropathy (MMN) that is most amenable to treatment, the presence of conduction block in motor nerves at sites not prone to compression and F waves with chronodispersion and impersistence may be found. In view of the unusual sites, conduction block may prove difficult to find. However, in any patient with an asymmetrical, pure, lower motor neurone disorder, it is important to carry out an extensive electrophysiological study, as a routine study may indicate a diagnosis of MND, which obviously carries a very different prognosis. Subsequent monitoring of sites where conduction block had been found may show improvement or reversal following treatment. The presence of markedly raised titres of antibodies against GM1 ganglioside may help in the diagnosis of MMN with conduction block (see section 1.4.1.6).

Behnia and Kelly (57) pointed out that early in the course of MND, widespread denervation may not be apparent and thus a definite diagnosis cannot be made. Following the results of the original clinical study using threshold electrotonus in patients with MND (21), it was hoped that a role might be provided for the technique as an early diagnostic tool, at a time when standard neurophysiological tests are found lacking.

A further limitation of standard neurophysiology in MND is the poor correlation of the available techniques with disease progression, as a steady clinical course does not imply a constant denervation-reinnervation ratio. Two types of abnormality (Type 1 and Type 2 - see later) were found when recording threshold electrotonus from patients with MND (21). Subsequent animal and nerve model experiments appeared to suggest a possible progression of potassium channel
dysfunction as an explanation for these abnormalities. It was hoped that by charting such a progression in patients with MND, that threshold electrotonus might provide a prognostic role in the disease.

1.4.1.5 Pathology of Motor Neurone Disease
The cardinal pathological features of MND are loss of spinal anterior horn cells, motor neurones in the lower cranial nerve nuclei and the giant pyramidal cells of Betz in the motor cortex, in addition to degeneration of the corticospinal tracts. In sporadic MND, involvement of the posterior columns is rare. However, up to 70% of cases of familial MND have degeneration of the posterior columns, although sensory symptoms are rare (59).

Immunohistochemical techniques have brought to light the presence of characteristic ubiquinated inclusion bodies within anterior horn cells, though not in motor cortex cells, which is a constant finding in sporadic, familial and Western Pacific forms of MND (60). They are not found in other neurodegenerative diseases, poliomyelitis or spinal muscular atrophy. The presence of filamentous structures within these inclusions may indicate that ubiquitin is associated with altered cytoskeletal components. However, unlike other neurodegenerative diseases, in MND, antibodies against cytoskeletal proteins do not identify the skein-like inclusions.

Recent studies have shown a glutamatergic deficit in MND (61). The cause for this is uncertain at present but possibilities include neuronal loss, reduced glutamate synthesis and altered distribution of glutamate with accumulation in the extracellular space, either due to decreased re-uptake or to loss of cholinergic inhibition of glutamate release.

1.4.1.6 Aetiological Theories
As the sub-title suggests, the aetiological mechanisms responsible for sporadic MND remain theories. Various factors that have been
implicated are toxins, ageing, trophic factors, viruses, metabolic abnormalities, autoimmunity, and oxidative stress.

Toxins
The possibility of toxins causing MND has been widely studied with few firm conclusions.

Among the heavy metals, lead has been particularly implicated. Despite the fact that lead can cause a reversible, MND-like disease with upper and lower motor neurone involvement, there has been no evidence from large studies to indicate that lead was a risk factor for sporadic MND (62).

In the Western Pacific islands of Guam and Rota, where high incidences of MND occur, the traditional food source, the cycad nut, is known to contain a neurotoxic amino acid, L-BMAA. However, the local practice is to wash the cycad nut flour extensively prior to ingestion, thereby leaving the residual flour substantially free from the soluble L-BMAA. Also, there is a delay of several decades following exposure to the cycad nut before symptoms develop.

Olney et al. established a strong correlation between the ability of acidic amino acids to excite neurones and to cause a characteristic neuropathology, both in vivo and in vitro and thus proposed the 'excitotoxic hypothesis of neuronal death' (63;64). Glutamate has been suggested as a possible excitotoxin and, as a result, the anti-glutamate drug, riluzole was trialled in MND (65;66), and has been found to increase survival. Whether it does so through its anti-glutamate actions, however, is uncertain. Evidence for the involvement of glutamate in the disease comes from many sources. Plaitakis and Caroscio (67) found significantly increased plasma glutamate in patients with MND. However, the significance of a raised plasma glutamate, in terms of the biochemical processes in the central nervous system, is probably small. Difficulties in the interpretation of cerebrospinal fluid (CSF) glutamate
are compounded by the complex metabolism of glutamate. The CSF glutamate has been found to be either unchanged (68;69) or significantly increased (70). Levels of glutamic acid (71) and glutamate (72) have been found to be reduced in the brain and spinal cord of patients with MND. It is possible that the abnormalities detected may result from neuronal death, but these investigators believe that a defect in glutamate handling is the primary pathogenic process. Based on this theory, a number of therapeutic trials have been undertaken, in addition to riluzole, and include branched-chain amino acids, MK801 and dextromethorphan.

**Ageing**

The natural process of ageing and the pathology of MND have a number of similarities and therefore the concept of 'premature ageing' has been proposed. Both processes involve neuronal loss in the ventral horns, a decreased motor neurone RNA content and a decrease in motor unit number measured electrophysiologically (73-75). No studies to date support the idea that MND can be simply equated with premature ageing, but since the disease typically presents at an age when motor neurones normally start failing in large numbers, it remains a possibility that ageing plays a significant role in the disease.

**Trophic Factors**

The importance of retrograde control of motor neurone structure and function is well documented. A two-fold excess of motor neurones are present in the developing vertebrate embryo, which subsequently die following failed competition with surviving motor neurones, for a trophic factor produced by muscle (76). Muscle-derived trophic factors have been shown to prevent motor neurone cell death (77). Motor neurone sprouting is likely to be controlled by a similar factor (78). The importance of muscle-derived factors is seen when loss of motor neurones follows amputation (79).
Abnormalities in the motor neurotrophic system may be part of a final common pathway for the development of motor neurone disease (80). However, there is no evidence to date that a specific deficiency in a motor system trophic factor exists, or that an immunological response occurs to a factor or factor receptor.

Viruses
Certain viruses are known to affect areas of the motor system, but none have been found to be linked causally with sporadic MND.

A type C murine RNA virus can cause a transmissible lower motor neurone disease without inflammation (81).

Then there is the clear relationship between a history of acute paralytic poliomyelitis many years previously and the development of progressive muscular atrophy ('post-polio syndrome'). Dalakas (82) studied the morphological, electrophysiological, virological and immunological aspects of post-polio syndrome and concluded that a continuing dysfunction is present in the spinal motor neurones, resulting in ongoing muscle denervation and reinnervation which is first evident at the axonal branch points. He felt that the symptoms are related to 'attrition of the oversprouting motor neurones which after a period of time cannot support all their axonal sprouts, resulting in failure of reinnervation'. The syndrome does not appear to depend on renewed or persistent infection (83), although some patients have the presence of defective viral particles in the CSF (82;84).

A further enterovirus has been implicated in MND. Woodall et al (85) found conserved enteroviral sequences closely related to coxsackie B virus sequences in the spinal cords from patients with sporadic MND and from one case with possible familial MND. However, no other authors have reported this finding.
Human T-lymphotrophic virus type I (HTLV-I), the causative agent in adult T-cell leukaemia /lymphoma, also causes tropical spastic paraparesis, which affects the corticospinal tracts but spares the anterior horn cells.

The absence of positive evidence for a viral aetiology, does not disprove it and further studies involving culturing viruses, viral serology, ultrastructural identification of viral inclusions and *in-situ* hybridisation continue to be undertaken.

**Metabolic Abnormalities**

Abnormalities of glucose metabolism have been proposed (86;87), as hypoglycaemia found with insulinomas may be associated with weakness and wasting of muscles (88). However, there is a lack of consistent abnormalities of glucose tolerance in patients with MND, thus limiting the significance of this theory (89;90).

Abnormalities of folate metabolism have also been proposed (91), which might explain the reduced neuronal RNA in MND patients (92-94). However the abnormalities that have been described have been shown to result in a neuropathological effect similar to subacute combined degeneration of the cord, with involvement of sensory neurones, in addition to corticospinal tracts, but without compromise of lower motor neurones (95;96).

Abnormalities in the metabolism of a number of other agents have been proposed, including androgens (97), thyrotrophin-releasing hormone (98;99), cyclic nucleotides (100), and dopamine (101). However, none have been shown to be a consistent finding in patients with MND.

**Autoimmunity**

An increased incidence of autoimmune disease (102) and paraproteinaemias (103) has been found in MND. There is no direct evidence, though, that paraproteins have specific reactivity with motor neurone constituents. Moreover, no clear histocompatibility (HLA) sub-
type has been consistently found in MND patients (104;105), nor have any consistent changes in cellular immunity be found (102).

The search for autoantibodies against neurones in sporadic MND has been extensive but with few conclusive results (106-111). Antibodies directed against GM1 ganglioside, which is one of the most abundant gangliosides in neuronal membrane, have been found in high titres in patients with lower motor neurone conditions with or without the presence of conduction block (multifocal motor neuropathy; MMN) (112-116). When conduction block is found, the disorder is particularly amenable to immunosuppressive agents (58). In sporadic MND mildly elevated titres of anti-GM1 antibody may be found but they have not been found in high titres and the resistance of MND to immunosuppressive treatment, refutes the direct involvement of these antibodies in the disease. Similar low levels of anti-GM1 antibody have also been found in a number of patients with other neurological diseases and in some normal subjects (116).

The development of two experimental models in animals suggests that an autoimmune mechanism may explain motor neurone destruction. The first, 'experimental autoimmune motor neuron disease' (EAMND), is a gradual onset, lower motor neurone syndrome, induced in guinea pigs by injection of bovine spinal cord motor neurones (117). The second, 'experimental autoimmune grey matter disease' (EAGMD), is a more acute disorder involving both upper and lower motor neurones induced in guinea pigs by spinal ventral horn homogenates (118). In EAGMD, inflammatory foci are found in the central nervous system with evidence of breakdown of the blood-brain barrier, thus suggesting a means whereby IgG might gain access to central cells and processes.

More recent studies have demonstrated the presence of antibodies to L-type (119;120) and P-type (121) calcium channels in patients with MND and that the titre of calcium channel antibodies correlates with the rate of progression of MND. Also, MND IgG has been found to be selectively
toxic to a motor neurone hybrid cell line and that the calcium-dependent
cytotoxicity can be blocked by antagonists of N-type and P-type calcium
channels and removed by preabsorption with whole purified voltage-
gated calcium channel or with the $\alpha_1$-subunit (122).

Nevertheless, the specific limitation of the autoimmune theory of MND
lies with the failure of immunosuppressive therapy or plasma exchange
to halt or reverse the disease process (123;124). There remains the
possibility that autoimmunity may initiate the disease and allow
subsequent motor neurone destruction through other mechanisms. For
instance, threshold to excitotoxic amino acids may alter (67;72), or there
may be an increase in the number of reactive microglia causing release
of destructive cytokines and superoxide radicals. In these situations the
effect of immunosuppressive therapy and plasma exchange would be
minimal.

**Oxidative Stress**

In 10 - 20% of cases of familial MND (or about 1% of all cases of MND),
there is a mutation of the gene encoding Cu,Zn superoxide dismutase
(50), which acts to remove harmful superoxide radicals. The possibility
that free radical might form the aetiological basis of MND has been
suggested. Free radicals could lead to neuronal death through lipid
peroxidation causing membrane destruction, damage to DNA repair
mechanisms or key neuronal proteins. Some evidence for the latter has
been found in sporadic MND with elevation of protein carbonyl content
by 85% compared to controls (125). However, there has not be found to
be any abnormality of Cu,Zn superoxide dismutase in patients will
sporadic MND and there is little other evidence to confirm the free
radical theory of causation.

1.4.1.7 Current Treatment Trials

Numerous agents have been trialled in the past with little positive result.
The following description of possible treatments refers to trials recently
completed or ones that are still in progress and relate to some of the aetiological theories described above. Future trials are likely to target glutamate antagonists, neurotrophic factors and drugs that protect against free radical damage.

**Branched Chain Amino Acids**

The trial of branched chain amino acids was based on the hypothesis that partial glutamate dehydrogenase deficiency can occur in atypical motor neurone disease and in forms of multiple system atrophy, and that branched chain amino acids activate glutamate dehydrogenase and may modify glutamate metabolism and glutaminergic transmission. The results of an early trial indicated a significant benefit in maintenance of muscle strength and walking ability in the 22 patients treated (126), but those from a larger double-blind, placebo-controlled trial found a higher mortality in the treatment group (127). The largest trial, involving multiple European centres and 400 patients found no difference in outcome in the treatment group (128).

**Glutamate Inhibition**

With evidence for altered glutamate handling in MND, numerous trials of glutamate inhibitors have been carried out, including dextromethorphan, lamotrigine, MK-801 and riluzole. Riluzole is discussed in more detail in Chapter 6. The other drugs have not shown any clear survival benefits to date.

**N-Acetylcysteine**

N-acetylcysteine is a precursor of glutathione which acts as a free radical scavenger. This seems to neither cause harm, nor produce any benefit (129).

**Neurotrophic Factors**

*Ciliary Neurotrophic Factor:* This is a neuroactive cytokine made in Schwann cells, which initiates repair processes and promotes survival of neuronal tissue cultures. However the systemic side effects of the recombinant preparation were too great in patients with MND, with no
evidence of benefit (130;131). However, intrathecal preparations have been better tolerated (132).

**Insulin-Like Growth Factor (IGF1):** This mediates the action of growth hormone and has been shown to reduce cell death of motor neurones in vivo during normal development or following axotomy or spinal transection (133). It also enhances motor neurone sprouting in vivo (133). Human recombinant IGF-1 (rhlGF-1) decreases muscle atrophy and promotes muscle function in animal models of motor neurone disease (134). Two large, prospective placebo-controlled trials have been performed with rhlGF-1 in North America and Europe. There appeared to be some survival benefit in one trial (135) but this was not replicated in the second (136).

**Brain-Derived Neurotrophic Factor:** This enhances the survival of motor neurones after axotomy and can protect developing motor neurones. Trials of the recombinant preparation had a negative effect when given subcutaneously.

### 1.4.2 Threshold Electrotonus and Motor Neurone Disease

In the initial clinical study testing the hypothesis that a possible widespread disturbance of the axonal membrane in MND could be further differentiated by threshold electrotonus (21), 11 patients with a diagnosis of definite MND were tested. The results were compared to 15 normal control subjects, 6 subjects with benign fasciculations and 25 patients with neurological conditions affecting the hands (6 with upper motor neurone conditions and 19 with a variety of lower motor neurone conditions). It was found that the motor axons of patients with MND, unlike those in the normal control or neurological control groups, responded abnormally to subthreshold depolarising currents by either becoming more (Type 1) (Fig. 1.3) or much less excitable (Type 2) (Fig. 1.4) than normal. Moreover, both types of abnormality could be reproduced in rat nerves *in vitro*, and in a computer model of human
motor axons, by reducing voltage-dependent potassium conductances. When the fast potassium channels were blocked in both the rat axon (with 4AP) and in the computer model, a pattern identical to the Type 1 abnormality seen in the MND patients was found to develop and represented the changes described previously in the S1 component of electrotONUS (section 1.3). When both the fast and slow potassium channels were blocked in the rat axon (with 4AP and TEA) and in the computer model, a pattern identical to the Type 2 abnormality seen in the MND patients was found to develop. This produced regenerative depolarisation and an abrupt fall in excitability. In the latter case, changes were seen in both the S1 and S2 components of electrotONUS.

The results of this study pointed towards an imbalance between functional sodium and potassium channels as a cause for fasciculations in MND, as opposed to those arising in normal subjects or those with root or peripheral nerve disorders. Also suggested was the possibility that the ion channel dysfunction could also be responsible for the motor neurone degeneration in the disease as the same results were not found in patients with other anterior horn cell conditions such as polio and spinal muscular atrophy.

From this original clinical study a number of potential clinical applications for the technique of threshold electrotONUS became evident.

First, there arises the possible role of the technique as a diagnostic tool for MND, since no other condition produced the abnormalities seen in MND. Currently, there is no definitive diagnostic test for MND. One relies on a combination of the clinical picture (e.g. El Escorial criteria), the EMG findings, which are not specific to the disease, (see section 1.4.1.4) and the absence of positive findings in other tests (e.g. normal imaging and normal or only slightly elevated anti-GM1 antibodies). Despite this wide range of available tests, one is often faced with a patient with upper and / or lower motor neurone signs in one or two regions, with the likely EMG findings and negative findings in all other
tests. However, a diagnosis of definite MND cannot be made until the disease has progressed sufficiently. In addition, all recordings made in the initial study were from abductor digiti minimi (ADM) with stimulation of the ulnar nerve at the wrist and it was therefore not known whether other nerves and more proximal stimulation would also produce similar results.

Secondly, Bostock et al (21) demonstrated that all their MND patients showed one of two abnormalities (Type 1 or Type 2). In vitro and computer models suggested that Type 1 changes indicated fast potassium channel dysfunction, whereas Type 2 changes indicated the combination of fast and slow potassium channel dysfunction. What was not demonstrated by the study was whether Type 2 changes represented a progression from a Type 1 pattern and thus a progression of the disease. They did point out however, that there was a strong impression that the Type 2 abnormality was seen in those patients that appeared to have a more rapidly deteriorating disease. Therefore, after the initial study it remained unclear whether the two different abnormalities represented progression of the disease or the rate of progression of the disease. What was required was to establish what the abnormalities actually represent by carrying out repeated testing over a prolonged period of time, and thus provide a possible role for threshold electrotonus as a prognostic tool in MND

Finally, the question arises that if potassium channel dysfunction and therefore, an imbalance between functional sodium and potassium channels, appears to be specific to MND (whether this occurs as a primary or secondary event), it may be possible to redress this imbalance with membrane stabilising agents and produce symptomatic relief and maybe even slow down or stop the progress of the disease. Recent trials of drug treatment in MND have focused on the excitotoxic
Figure 1.3: An Example of a Type 1 Abnormality

Threshold electrotonus waveform from a subject with a type 1 abnormality (black trace) superimposed on the waveforms from the normal control group (grey dotted traces). Threshold changes (top) due to the polarising currents (bottom) are expressed as percentages of the threshold current tested with 1ms pulses. The responses to depolarisation are represented as positive threshold reduction (0 to 100%) and the responses to hyperpolarisation are represented as negative threshold reduction (0 to -100%) in order to correlate with standard electrotonus traces. In a type 1 abnormality the responses to hyperpolarisation fall within the normal range, but the depolarisation responses exhibit heightened excitability (increased threshold reduction) maximal between 10 - 20 ms delay.
Figure 1.4: An Example of a Type 2 Abnormality

Threshold electrotonus waveform from a subject with a type 2 abnormality (black trace) superimposed on the waveforms from the normal control group (grey traces). Threshold changes (top) due to the polarising currents (bottom) are expressed as percentages of the threshold current tested with 1ms pulses. In a type 2 abnormality the responses to hyperpolarisation fall within the normal range, but the depolarisation responses a dramatic reduction in excitability (reduced threshold reduction) maximal between 10 - 20 ms delay.
theories of aetiology, with excess glutamate as the cause. The drug riluzole, part of the benzothiazole family of compounds, is now licensed for use in patients with MND, as it has been shown to prolong survival in the disease (65;66). It acts primarily by altering glutamate release, which is the reason it was trialled initially, however, it also has an effect on voltage-gated sodium channels, causing inactivation of these channels. It may therefore follow from the hypothesis given by Bostock et al. (21), that the reason riluzole has an effect on survival in MND is that it produces stability of the axonal membrane by redressing the sodium - potassium imbalance, rather than through its anti-glutamate action. It is therefore possible that threshold electrotonus recordings may be able to detect the improvement by reversal or amelioration of Type 1 or Type 2 abnormalities. It is also possible that other membrane stabilising drugs, primarily anticonvulsants such as carbamazepine and phenytoin, may have a similar effect. These particular drugs, though in regular use for epilepsy, have not been trialled in MND. The use of threshold electrotonus recordings in conjunction with these drug therapies, may therefore provide a monitoring role for the technique, as well as opening avenues for potential future therapeutic trials.

In order to provide answers to these questions the current study was devised. The aims of the study were:

1) To assess the reproducibility of the previous findings (21) and ascertain the specificity and sensitivity of the technique of threshold electrotonus as a diagnostic tool in MND. This was performed by making multiple recordings, from different sites along the same nerve and from different nerves, in a larger number of patients with MND and comparing them to normal controls and a wide range of patients with other neurological diseases.

2) To establish whether the technique could be used prognostically in MND by making longitudinal studies in comparison with other tests of motor neurone function, and determining whether the development
and progress of the channel dysfunction is consistent with its proposed role in pathogenesis.

3) To assess the effect of membrane stabilising medication on threshold electrotONUS traces, to ascertain whether a reversal or amelioration of abnormal recordings was possible and thus a possible monitoring role for the technique. With its current widespread use and effects on improved survival in MND, riluzole, which also has actions on sodium channels, was the most obvious candidate. It was hoped to trial other membrane stabilising medications, specifically carbamazepine and phenytoin, which are not licensed for use in MND. Ethics committee approval was granted, but patients who fitted the inclusion criteria were limited. Ultimately only one patient was recruited to take carbamazepine.
2. METHODS AND SUBJECTS

2.1 INTRODUCTION

The study of the clinical applications of the technique of threshold electrotonus required recordings to be made from a number of different groups of subjects, under various conditions and with certain modifications made to the apparatus and the computer software. The results of these studies are presented in the following chapters, while details of the general apparatus, the standard threshold electrotonus technique and principles of patient and control selection are presented in this chapter.

2.2 GENERAL EQUIPMENT

The technique of threshold electrotonus requires the use of long duration, subthreshold polarising currents (100ms). Currently available commercial stimulators are unable to provide such long duration pulses. Therefore, an isolated stimulator was designed by Hugh Bostock from the Sobell Department of Neurophysiology in the Institute of Neurology and Peter Fitch from Electronics division of the Department of Clinical Neurophysiology, in the National Hospital of Neurology and Neurosurgery (NHNN). The equipment was built by Gareth Bahlke, also from the Electronics division at the NHNN. The stimulator functions as a bipolar voltage to current converter with a maximal output of 50mA (isolated). The stimulator was designed to be as lightweight and as portable as possible to enable recordings to be made from patients in their homes and at other hospitals. A suitcase on wheels was adapted to house the stimulator to facilitate transportation.
A computer was used to generate stimuli. An IBM 486 PC was used when patients were seen in the laboratory. For recordings made outside the laboratory, a laptop (Cardstar 3000) was used. The current source was controlled by the computer via a data acquisition board (Data Translation DT2812). The amplified signal was filtered (1 Hz to 5 kHz) and sampled at 10 kHz.

2.3 SOFTWARE

No publication to date about the use of the technique of threshold electrotonus has described the operation of the programme in detail. Therefore the following section will provide such a description for those unfamiliar with the technique.

2.3.1 Data Acquisition

Recording protocols, with prompts, were written for the threshold tracking programme (QTRAC v 1.5, 1.6, 1.8, copyright Institute of Neurology). Modifications were made over the period of study to enable simplification of the technique. QTRAC is a flexible, stimulus-response data acquisition programme, which enables threshold-tracking and averaging for experiments in which excitability or the response of the preparation varies slowly with time. Changes in selected parameters of the response can be accessed through the programme e.g. threshold, latency, amplitude etc. All or selected raw data can be recorded and regenerated off-line to provide a check on successful tracking and also allow calculation of other parameters, such as initial threshold. The programme has separate stimulation and plotting sub-divisions, but these share the mode of operation and many of the same facilities.

The colour display facility within the programme obviates the need for ancillary display devices such as oscilloscopes and thus reduces the number of pieces of equipment, which was especially important for the
development of a portable system. Any combination of stimulus waveform, response waveform, whether raw or modified, or other measured parameter can be displayed or rescaled at any time during a recording.

QTRAC is also a multi-channel programme, in which up to 10 channels may be associated with different physical inputs and outputs, various stimulation parameters or diverse operations on the response waveform.

The programme is operated via a keyboard. Any parameter can be changed and these are automatically logged. Prompts are available throughout to facilitate any such changes and additional comments can be typed in.

Data gathered from recordings can be printed in black and white or colour on most standard printers using the shareware utility PCAD.

2.3.2 Stimulation

Stimulation and recording are effectively simultaneous and can be sampled between 1 and 50kHz. As indicated above, for the following experiments, sampling was carried out at 10kHz. The stimulus waveform is made up of a Test stimulus, whose height can be varied depending on the response to effect threshold tracking. The programme allows a number of different shaped Test stimuli, however for the purposes of these experiments rectangular pulses were used. Conditioning stimuli can be added to the programme and set to a fraction of the Test stimulus, as was required to enable the testing of electrotonus. These additional features are added on separate channels. Moreover, the programme allows the combination of a Test and Conditioning stimulus, which can be individually advanced or retarded in specified regular steps, with a measure made of the Test-Conditioning interval. Thus in the majority of our experiments 5 channels were utilised, the first with a Test pulse alone and the remainder with the Test pulse superimposed upon long duration pulses set to different
fractions of the Test pulse and these channels were alternated. These latter features form the crux of the technique of threshold electrotonus, enabling the measurement of the response of axons to defined sub-threshold polarising or Conditioning currents at set intervals during and after a Test stimulus.

2.3.3 Response Measurement

The QTRAC programme allows modification of the raw responses by filtering and the baseline can be clamped to zero before, or before and after the 'window' to correct for a sloping baseline. The window is used to determine the section of the response to be measured. Once a response, either a compound muscle action potential (CMAP) or sensory action potential (SAP), has been produced following initial stimulation of the nerve, the starting time and duration of the window are entered via the keyboard. A line on the display indicates the window. For instance a classical CMAP, produced from stimulation of the ulnar nerve at the wrist and recorded from ADM, starts at approximately 5ms after the stimulus and lasts about 10ms. Therefore, the window, or the period during which the computer measures the peak response would stretch from 5ms to 15ms. The baseline could be clamped on either side, if required, although for measuring CMAPs the baseline is usually only clamped before the response.

In addition, it is essential to the technique of threshold electrotonus to set the window height or target amplitude of the response, which is set at a fraction of the maximal response. This is determined from baseline to the peak for CMAPS and from peak-to-peak for SAPs. In original versions of QTRAC the window height was set manually at 40% of the maximal amplitude of the response to stimulation. Later versions have incorporated an automatic system once a supra-maximal response has been achieved. However, this can be overridden to allow recordings to be made at different percentages of the maximum amplitude. Also incorporated into later versions is the ability to set a percentage error.
acceptable for the response with respect to the window height. In other words responses with amplitudes within, for instance +/- 10% of the specified height are accepted without further trials and thus the recording time is reduced.

The latencies are also measured. They can be taken from the stimulus to positive peak, half peak or an arbitrary level, or the width at half peak height can be recorded. As part of the display, lines indicate which latencies and peaks have been measured, but these can be changed.

2.3.4 Threshold Tracking

In the technique of threshold electrotonus, contrary to historical tests of the measurement of accommodation, the amplitude of the response is set and the amplitude of the Test stimulus is altered. Thus, the programme allows the amplitude of the Test stimulus to be increased or decreased by a specified percentage after each response, depending on whether the peak, or peak to peak, value within the window exceeded or fell below the specified threshold. The step size can be kept constant or automatically adjusted within limits to provide improved tracking when there are rapid changes in excitability. The recent tracking history of each channel is displayed separately, indicated by a ‘+’, ‘-‘ or ‘=’ sign.

2.3.5 Display

The screen can be split into up to eight plots, which can be altered at any time. The choice includes: Stimulus waveform, Raw response, Modified response (i.e. after filtering and clamping), Threshold, Latency, Peak, Conditioning-Test delay, Conditioning current at the time of the Test pulse (ipOl) and slowly-sampled A/D inputs (Fig. 2.1). Traces from the different channels are shown in the same plot using colour coding to differentiate them. However they can be displayed independently in separate plots.
Moreover, from the plotting sub-division of the programme (QTRACP), additional User-defined channels allow plotting of values calculated from any combination of measurements recorded. This includes any comments that are typed in the form of numbers. For instance, one can plot skin temperature, which has been typed in as comments during a recording, against threshold reduction at a particular delay for a particular channel.

Any displays that are produced can be saved as a file or printed.

2.3.6 Files Generated by the Programme

QTRACS generates three types of files which are used for threshold electrotonus, with extensions .QZD, .QRP and .QTG.

The QZD files are data files, which store the following values for each test: the channel number; threshold (Test stimulus amplitude); measured peak height and latency; conditioning-test interval and the conditioning current. Any comments that are typed in during the recording and parameter changes are also saved at their appropriate time points. Scaling factors and other such parameters are specified as the file is closed.

The QTG files save individual raw response traces, but have the same initial name as the corresponding QZD file. Each threshold electrotonus recording produces a large amount of data and thus fills the hard drive of a PC relatively rapidly. Thus separating the data into smaller QZD and larger QTG files enables one to download the information on to optical disks, but keep the QZD files on the PC for quick reference. Should more detailed analysis be required, then both files can be accessed through an optical disk drive. The QZD and QTG files are fully cross-referenced and thus when they are together, either prior to downloading on a PC or through the optical disk drive, they appear to the user as a single file.
The QRP files contain the recording parameters for each test. They also contain any instructions that have been typed during a recording that have changed any parameters. They therefore define the experimental protocol and can be edited by a text editor to restart an experiment with altered parameters.

The file names are generated automatically. They are made up of a prefix letter (which was ‘F’ in our recordings), the date in reverse as a six digit number and a one letter run code which is automatically increased from A-Z (i.e. the first test is ‘A’, the second test is ‘B’ etc.).

2.4 THRESHOLD ELECTROTONUS RECORDINGS

The general principle of the method of threshold electrotonus is illustrated in Figure 2.2. The application of the method in a clinical context was first described by Bostock et al (21).

All recordings utilised the same basic protocol, however some individual experiments required minor changes in either the software programmes or ancillary equipment, and these will be described separately in the relevant chapters.

The positioning of electrodes was the same as is carried out for standard nerve conduction studies. The nerves were stimulated via a non-polarising electrode (‘Red-dot’: 3M Canada Inc., London, Ontario, Canada) with a remote indifferent electrode. A Red-dot electrode was also used as an earth. Self-adhesive recording electrodes were placed on the relevant muscle (Dantec 9013L202 surface electrodes). Sensory studies in the hand utilised muscle stimulus electrodes (Myocare 3M 6281), which were cut into narrow strips to form ring electrodes and the nerve was stimulated antidromically with recordings made from the relevant finger.
Figure 2.1: Threshold Electrotonus Stimulus and Response Parameters

Recordings from five different stimulus conditions superimposed showing the possible combination of parameters that can be displayed on the computer screen. Thick lines represent the subthreshold depolarising current set to 40% of the control threshold current. (A) Five consecutive stimulus waveforms superimposed, comprising 100ms polarising and 1ms test stimuli (depolarising current upwards). (B) Raw EMG responses corresponding to stimuli in A. (C) EMG responses modified by baseline clamp and digital smoothing, for measurement of peak height. The horizontal line indicates window duration and target response amplitude. (D) Changes in peak stimulus current, showing tracking of changes in excitability caused by polarising currents (depolarising current initially downward - thick line). (E) Delay between the start of the polarising stimuli and that of the test stimulus. (F) Amplitude of the polarising current (measured at the start of the test stimulus), set to 40%, 20%, -20% and -40% of the last control test stimulus. (G) Changes in EMG peak response amplitude, showing how this was stabilised by threshold tracking.

[Figure taken from Bostock, Sharief, Reid and Murray, Axonal ion channel dysfunction in amyotrophic lateral sclerosis. Brain 1995; 118: 217-225].
The majority of the tests were carried out on motor fibres and the following description of how threshold electrotonus recordings were produced will focus on the method for motor nerves. However the method for producing sensory threshold electrotonus recordings is identical except for the initial acquisition of a sensory nerve action potential and the measurement of peak-to-peak amplitude rather than baseline to peak.

Initially, once the electrodes are positioned, the QTRAC programme is begun and a raw baseline trace appears on the screen to ensure that there is no extraneous electrical noise. The nerve is then stimulated by a 1 ms, 1 Hz test pulse which is stepped up to produce a maximal compound muscle action potential (CMAP). A stimulus-response curve is also recorded by the computer. The start of the window (i.e. latency to the start of the CMAP) and its length, are entered via the keyboard. In earlier programmes the fraction of the peak height (e.g. 40% of the maximal amplitude) was also entered manually, but subsequent programmes automatically adjusted the stimulus at this stage to produce an amplitude that is 40% of maximal via electronic feedback. It is however possible to override this automatic adjustment if a different fraction of maximal was required.

Nerve excitability is then tested by alternating up to four levels of polarising currents of 100ms duration, which are superimposed on the 1 ms test pulse. The nerve is stimulated at 1 Hz. Up to five stimulus conditions are tested in turn throughout the test: the 1 ms test alone (control) and the test stimulus superimposed on the 100 ms polarising currents set to 20%, -20%, 40% and -40% of the last control stimulus.

The “threshold” is subsequently tracked to maintain the pre-set amplitude of the response waveform. The time interval between the polarising pulse and the test pulse is altered such that the polarising current started 2 ms after the test stimulus at the start of the programme and is stepped up to 198 ms before the test stimulus as the
programmed progresses. The test stimulus is always applied at 201 ms after the start of the sweep.

Thus the polarising stimuli are kept at a fixed amplitude with respect to the last test pulse (40%) but their relative timing with respect to the test pulse is altered. The test pulse is fixed in its timing (201 ms), but its intensity is modified to produce a constant CMAP amplitude.

Excitability changes due to the polarising currents are expressed as a 'percentage reduction' of the control threshold, allowing for a dimensionless parameter to facilitate the comparison of different nerves. The interval between the polarising and test pulse is used as the abscissa for the threshold electrotonus plots. All the waveforms displayed during the recordings are saved on optical disk (Panasonic WORM 1.4 GB), including the raw EMG data, and could be regenerated off-line to check on the tracking and allow measurement of other parameters.

In earlier programmes a full threshold electrotonus recording from one nerve would take 20 minutes for five channels (i.e. control plus test pulse superimposed on 20%, -20%, 40% and -40% polarising currents) and 10 minutes for three channels (i.e. control plus test pulse superimposed on 40% and -40% polarising currents). Subsequently, programmes were written to speed up the process by accepting responses with up to 10% error and by increasing the rate of tracking, which proved to produce comparable recordings and allowed tests to be completed in 4 minutes for five channels and 2 minutes for three channels.
Figure 2.2: Schematic Diagram Illustrating the Principle of Threshold Electrotonus Recording from a Motor Nerve

The computer carries out functions within the dashed box. Compound muscle action potentials (CMAPs) recorded from abductor digiti minimi (ADM) are compared with a target response set at 40% of the maximal response. An error signal is used to alter the amplitude of the 1ms test stimulus pulse, which is applied to the ulnar nerve at the wrist via an isolated voltage-to-current converter. The test stimulus is combined, at varying delays, with a 100ms subthreshold depolarising or hyperpolarising stimulus and the response is recorded, completing the feedback circuit.

[Figure taken from Bostock, Sharief, Reid and Murray, Axonal ion channel dysfunction in amyotrophic lateral sclerosis. Brain 1995;118: 217-225].
2.5 SUBJECTS

All patients were recruited with informed consent and were selected from The National Hospital for Neurology and Neurosurgery, The Maudsley Hospital, Charing Cross Hospital and St Thomas's Hospital with the permission of the consultants in charge of their care. All patients had a history and examination carried out and details of relevant investigations, such as neurophysiology, blood tests and imaging, were recorded. Latterly skin temperature recordings were made on each patient. The study was approved by the Joint Hospital and Institute Ethics Committee.

The largest patient group and the main focus of the study consisted of patients with motor neurone disease. 70 patients with MND were studied in total (age range 31 - 84 years; mean age 60.2 years). The whole group was further subdivided into various sub-groups depending on clinical features. Those patients with both upper and lower motor neurone signs in two or three spinal regions or in the bulbar region and at least two other spinal regions were classified as 'amyotrophic lateral sclerosis' or 'ALS'. They therefore fell into the 'definite' or 'probable' categories of the El Escorial classification (56) (see Table 1.1). Those with purely bulbar features or predominantly bulbar features, with upper or lower motor neurone signs in one other region, were classified as 'bulbar'. Whereas, those with predominantly lower motor neurone signs were termed 'progressive muscular atrophy' or 'PMA' and those with predominantly upper motor neurone signs, 'primary lateral sclerosis' or 'PLS'. In addition, one patient with familial motor neurone disease, who was positive for the superoxide distmutase-1 (SOD-1) gene mutation, was studied. Although he fitted the criteria for definite ALS, he was classified within a separate group (SOD-1 +ve), to assess whether any differences emerged.
By sub-dividing the MND group in this way, the following patient groups emerged:

1) 45 patients with Amyotrophic Lateral Sclerosis (ALS) - 'definite' and 'probable' cases of MND as assessed by the El Escorial (56) criteria (age range 31 - 84 years; mean age 60.3 years).

2) 10 patients with Bulbar or Pseudobulbar Palsy ("Bulbar group") not found to have any other aetiology (age range 44 - 75 years; mean age 62.3 years).

3) 9 patients with Progressive Muscular Atrophy (PMA) with normal anti-GM1 antibody levels, no evidence of conduction block and no evidence of polyradiculopathy (age range 44 - 73 years; mean age 62.3 years).

4) 5 patients with Primary Lateral Sclerosis (PLS) not found to have any other aetiology (age range 33 - 73 years; mean age 50.4 years).

5) 1 patient with Familial MND who was positive for the SOD-1 gene (SOD+ve) (aged 37 years).

In addition to the history and examination being carried out and details of all investigations recorded, a number of patients with MND had repeated standard neurophysiological tests along with the longitudinal threshold electrotonus studies. Latterly, all patients were scored using a modification of the Appel Rating Scale for Amyotrophic Lateral Sclerosis (137) (see Appendix 1). The modifications were made to prevent the need for further equipment, as the majority of recordings made on the patients with MND were carried out in their homes using the portable system. Therefore modifications were made to the respiratory section to avoid the use of a spirometer, to the section on grip strength to avoid the use of a dynamometer and various components of the muscle function section that required additional equipment.

Of the 70 patients with MND in all sub-groups, all had threshold electrotonus recordings made from ADM, stimulating the ulnar nerve at
the wrist, to enable comparison with the initial study. The majority also had recordings from motor axons made with proximal stimulation of the ulnar nerve above the elbow, recordings from the contralateral arm and some from nerves in the leg. In addition, 37 patients had repeated tests every two to three months for as long as possible over the two year period of the study (22 in the ALS group; 7 in the Bulbar group; 4 in the PMA group, 3 in the PLS group and the 1 patient with SOD+ve MND). Of the 37 patients included in the longitudinal study, 8 were also tested using a single unit protocol.

A separate study was also undertaken to ascertain whether excitability changes as tested by threshold electrotonus were apparent in patients with multiple sclerosis (MS) (138;139), as had been found in two out of the four MS patients used as neurological controls during initial clinical study (21). 48 patients from the wards and out-patient department of the National Hospital for Neurology and Neurosurgery were selected on the basis of a diagnosis of clinically definite MS with laboratory support (age range 26 - 63 years; mean age 40.1 years). Conventional neurophysiological tests were carried out on those patients who had symptoms that might have been attributable to a root or peripheral nerve disorder. Ten out of the 48 MS patients were tested on at least two separate occasions, a month apart and urine and serum samples were collected at each test to assess the levels of various inflammatory parameters (See chapter 8).

64 patients (not including the patients with MS) with a wide range of upper and lower motor neurone conditions were also studied to act as neurological controls for the study group with MND (age range 13 - 77 years; mean 49.2). This group included patients with benign fasciculations (11), polio (15), spinal muscular atrophy (SMA) (7), multifocal motor neuropathy (MMN) (2), ulnar neuropathy (4), neuralgic amyotrophy (3), chronic inflammatory demyelinating polyneuropathy (CIDP) (6), peripheral sensory neuropathy (4), Parkinson's disease (1),
acute nerve injury (1), myopathy (1), multisystem atrophy (MSA) (1),
cervical myelopathy (2), neuromyotonia (2), late onset myotonia (1),
generalised dystonia (1), Friedrich's ataxia (1) and cerebrovascular
accident (CVA) (1). Of this group of 64 neurological controls, 8 patients
were followed up longitudinally (1 with polio, 1 with benign
fasciculations, 1 with SMA, 1 with an ulnar neuropathy, 1 with CIDP, 1
with MMN, 1 with neuromyotonia and 1 with a CVA).

The normal control group comprised 35 members of staff at The
National Hospital and The Institute of Neurology with no record of
neurological pathology (age range 22 - 60 years; mean age 36.5 years).

2.6 STATISTICAL ANALYSES

The threshold electrotonus data was plotted and analysed with
programmes already written for the purpose by Hugh Bostock.

Further statistical analyses were performed using the statistic functions
in Microsoft Excel for Windows, version 5.0.

Student t-tests and analysis of variance (f - tests) were used to assess
inter - and intra - patient variability. Correlation coefficients were
calculated to assess the relationship between threshold reduction and
pro - inflammatory factors in patients with multiple sclerosis (MS).

Statistical significance was taken as p < 0.05.
3. RESULTS - SINGLE RECORDING MOTOR NEURONE DISEASE STUDY

3.1 THRESHOLD ELECTROTONUS DATA

Excitability changes induced by the subthreshold polarising currents are expressed as a percentage threshold reduction or in other words, the percentage of the control threshold tested with the 1 ms test pulse. The interval between the two current pulses is used as the abscissa for the threshold electrotonus plots. Below each plot of threshold electrotonus recordings, the polarising currents used are represented as a percentage of the control threshold. An example of such traces can be seen in Figure 3.1.

Using this format, traces similar to those produced by classical electrotonus experiments, are produced. The parts of the traces with positive threshold reduction represent the excitability changes induced by depolarising currents, and those with negative threshold reduction represent the excitability changes induced by hyperpolarising currents.

All subjects tested had at least one recording made by stimulating the ulnar nerve at the wrist and recording from ADM, in order to provide a comparison with the initial clinical study (21). Most also had recordings made from the opposite wrist or from above the elbow. However, with the constraints on patients' time, only those that were seen on numerous occasions had recordings made from other sites or on sensory nerves. Therefore the majority of the data gathered pertains to the results from the motor testing of the ulnar nerve at the wrist.
Figure 3.1: Threshold Electrotonus Recordings From the Normal Control Group (Ulnar Nerve - Wrist Stimulation)

**A:** Superimposed threshold electrotonus waveforms from 35 normal control subjects (dotted lines). Responses recorded from ADM following stimulation of the ulnar nerve at the wrist. Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. Responses to depolarisation are represented as positive threshold reduction (0 to 100%) and those to hyperpolarisation by negative threshold reduction (0 to -100%).

**B:** Mean normal control threshold electrotonus waveform +/- one standard deviation.
Further analysis of data in the following sections will also focus on the responses of the various groups to depolarising currents between 10 and 20 ms delay when stimulating the ulnar nerve at the wrist, as this was the delay and site of stimulation at which the abnormalities were previously found in patients with MND (21).

3.1.1 The Rationale for Amplitude Percentage

Central to the technique of threshold electrotonus is the necessity to use subthreshold polarising currents that are set to a percentage of the control threshold. All tests that are subsequently reported utilised a peak height that was set to 40% of the maximal amplitude of the response in the control situation.

Although peak heights set to percentages lower than 40% produced no problems in normal control subjects or in neurological controls with good muscle bulk, when such settings were made in patients with marked wasting, the signal to noise ratio proved unacceptable.

When percentages higher than 40% were used, the situation often arose where such high levels of current were no longer subthreshold and action potentials were generated by the polarising currents. Figure 3.2 demonstrates this phenomenon in a patient with MND, in whom the peak height was set to 60% of the maximal CMAP amplitude. A clear response is seen to arise following the depolarising current, as well as following the test pulse and thus producing an inaccurate and bizarre threshold electrotonus recording.
Figure 3.2: The Use of Polarising Currents Set to 60% of the Maximal Compound Muscle Action Potential.

An illustration of how the use of polarising currents set to 60% of the maximal compound muscle action potential in a patient with MND, produces an inaccurate threshold electrotonus recording. The depolarising current is no longer subthreshold and generates a response. **A:** The raw EMG trace showing a single stimulus situation involving a combination of depolarising and test stimulus, at delay of 80 ms. Following the onset of the 100ms depolarising pulse, a response is seen and therefore the response following the 1ms test pulse does not represent the combined depolarising-test stimulus which is essential for the accurate measurement of threshold electrotonus. **B:** Changes in peak stimulus current caused by polarising currents throughout the course of the recording. The changes caused by the depolarising current are abnormal (see Fig. 2.1D). **C:** Delays between the starts of the polarising and test stimuli. Vertical lines in B and C represents the point at which A is taken.
3.2 NORMAL CONTROL DATA

3.2.1 Motor Recordings

3.2.1.1 Ulnar Nerve (Wrist-ADM)

Figure 3.1A shows the superimposed threshold electrotonus traces from the 35 normal control subjects, stimulating the ulnar nerve at the wrist and recording from ADM whereas Figure 3.1B represents the mean waveform +/- one standard deviation (SD). The waveforms correspond to previous single motor unit and CMAP recordings from normal subjects (1;21), and reveal a tight range of responses to depolarisation and a more widespread range of responses to hyperpolarisation.

The mean threshold reduction, in response to depolarisation in the normal control group, at 10 - 20 ms delay (the delay at which the abnormality was previously found in MND patients), was 66.9% and the threshold reduction at this delay ranged between 54.7 and 75.1%.

3.2.1.2 Ulnar Nerve (Above Elbow - ADM)

The pattern of the threshold electrotonus waveforms derived from stimulating the ulnar nerve above the elbow and recording from ADM is slightly different to stimulating at the wrist, producing a slightly flatter response to depolarising currents and a less fanned out response to hyperpolarising currents (Fig. 3.3A). Figure 3.3B compares the mean waveforms of above elbow and wrist stimulation, illustrating the slightly 'squashed' appearance of the above elbow traces.

3.2.1.3 Other Motor Recordings

Recordings were also made from stimulation of other motor nerves including the median nerve at the wrist, recording from abductor pollicis brevis (APB), the peroneal nerve at the ankle and fibular head, recording from extensor digitorum brevis (EDB) and the posterior tibial
Figure 3.3: Threshold Electrotonus Recordings From the Normal Control Group (Ulnar Nerve - Above Elbow Stimulation)

A: Superimposed threshold electrotonus waveforms from 35 normal control subjects. Responses recorded from ADM following stimulation of the ulnar nerve above the elbow. Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. Responses to depolarisation are represented as positive threshold reduction (0 to 100%) and those to hyperpolarisation by negative threshold reduction (0 to -100%). B: Mean normal control threshold electrotonus waveform comparing stimulation at the wrist (thin line) and above the elbow (thick line).
nerve at the ankle, recording from abductor hallucis (AH). Each nerve and site produced a slightly different waveform pattern (Fig. 3.4), although all follow the same basic pattern seen in the classical electrotonus experiments (Fig. 1.1) and those found when recording from the ulnar nerve.

3.2.2 Sensory Recordings

Testing sensory nerves with antidromic stimulation of the ulnar nerve at the wrist and recording from the fifth digit (F5), produce responses to depolarisation that are extremely similar to those from stimulation of the motor fibres at the same site. However there is a significant difference in response to hyperpolarisation between sensory and motor nerves (Fig. 3.5), with the emergence of the inward rectifier, discussed in Section 1.3, producing an upward 'sag' in the trace from 50ms delay, and as a result an exaggerated overshoot (post – hyperpolarisation hyperexcitability).

Sensory recordings were not undertaken on the motor neurone disease patients for a number of reasons. First, the disease process involves the motor system and no abnormalities in sensory recordings had been found to date. Secondly, recordings other than motor recording of the ulnar nerve were only carried out on patients that were followed up. Thirdly the recording of sensory potentials requires a very high signal: noise ratio, and as the majority of follow – up recordings were carried out in the patients' homes, this proved difficult to achieve.

3.3 MOTOR NEURONE DISEASE DATA.

3.3.1 Motor Recordings

3.3.1.1 Ulnar Nerve (Wrist-ADM)

Figure 3.6A shows the superimposed traces from all the MND patients stimulating the ulnar nerve at the wrist and recording from ADM and the
mean threshold reduction +/- one standard deviation, is shown in Figure 3.6B. The majority of the waveforms in figure 3.6A are similar to those in the normal control group, however the range is greater in the responses to depolarisation, maximal at between 10 - 20 ms delay (Fig. 3.7)

The mean threshold reduction, in response to depolarisation in the whole MND group, at 10 - 20 ms delay was 67.8% and therefore similar to the normal control group. However, the threshold reduction at this delay ranged between 9.5 and 87.3.1%. The divergence of the standard deviation lines at this delay (Fig. 3.6B), in addition to the distribution seen in figure 3.7, illustrate this point.

3.3.1.2 Ulnar Nerve (Above Elbow - ADM)

The recordings made from ADM in the MND patients stimulating the ulnar nerve above the elbow are compared to those made from normal controls in figure 3.8A and B. The mean above elbow traces from the normal controls and the MND patients are almost identical (Fig 3.8C), but again the range of responses is greater in the MND group, especially at 10 - 20 ms delay in the depolarising direction (Fig. 3.9).

3.3.1.3 Other Motor Recordings

Of the 35 patients tested on numerous occasions, 11 patients had motor recordings made from sites other than the ulnar nerve. Figure 3.10 compares the mean traces taken from these recording sites in the MND patients with the range of normal control traces.

All mean traces from the alternate sites in the MND patients fell within the normal control range and followed similar waveform morphologies.

In some of the MND patients, small hand and feet muscles were often found to be markedly atrophic and thus prevented threshold electrotonus recordings being made from them. Therefore, tests were carried out from less routinely used sites, such as stimulating the ulnar
Figure 3.4: Threshold Electrotonus Recordings From the Normal Control Group - Examples of Other Nerves Tested.

Mean normal control threshold electrotonus recordings from nerves other than the ulnar nerve (black lines) superimposed on the range of normal control responses from the ulnar nerve, stimulating at the wrist and recording from ADM (grey lines).  

**A:** Median nerve - stimulating at the wrist and recording from APB.  

**B:** Common peroneal nerve - stimulating at the fibular head and recording from EDB.  

**C:** Common peroneal nerve - stimulating at the ankle and recording from EDB.
Figure 3.5: Threshold Electrotonus Recordings From the Normal Control Group - Comparing Sensory and Motor Fibres

Mean normal control threshold electrotonus recordings from the ulnar nerve following stimulation at the wrist comparing motor responses recorded from ADM (grey lines) with sensory responses recorded from the fifth digit (dark lines). The response to depolarisation is similar for motor and sensory recordings. However, the response to hyperpolarisation in the sensory studies produces an upward 'sag' in the trace, from 50 ms delay, caused by an inward rectifier.
nerve above the elbow and recording from flexor carpi ulnaris (FCU) and stimulating the peroneal nerve at the fibular head and recording from tibialis anterior (TA). These sites were used as a means of following the progress in the individuals concerned, rather than to characterise the waveforms from these sites compared to normal subjects.

3.4 NEUROLOGICAL CONTROL DATA

The data pertaining to the patients with multiple sclerosis (MS), is presented in full in Chapter 8.

Of the remaining 64 patients with other neurological conditions, 8 were followed up on more than one occasion and thus the majority only had recordings made from the ulnar nerve. The entire group had recordings made by stimulating the ulnar nerve at the wrist and recording from ADM. Most also had recordings from the opposite wrist and from stimulation above the elbow. 2 of the 8 patients who had multiple recordings had tests carried out on the leg. No patients in this group had sensory recordings.

3.4.1 Motor Recordings

3.4.1.1 Ulnar Nerve (Wrist-ADM)

Figure 3.11A shows the superimposed traces of all the neurological control patients and Figure 3.11B, the mean trace from the neurological controls +/- one standard deviation. The range of responses, especially between 10 - 20 ms delay, is extremely similar to those of the normal controls (Fig. 3.1) and does not show the increased range seen in the MND group (Fig 3.6).
Figure 3.6: Threshold Electrotonus Recordings From The Whole MND Group (Ulnar Nerve - Wrist Stimulation)

A: Superimposed threshold electrotonus waveforms from 70 patients with MND. Responses recorded from ADM following stimulation of the ulnar nerve at the wrist. Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. B: Mean threshold electrotonus waveform from the whole MND group +/- one standard deviation.
Figure 3.7: The Range of Responses at 10 -20 ms Delay Comparing The Whole MND and The Normal Control Groups (Ulnar Nerve - Wrist Stimulation)

Percentage threshold reduction at 10 - 20 ms delay comparing recordings from 70 patients with MND (right) and 35 normal control subjects (left). The mean percentage threshold reduction is represented by the solid horizontal lines. The dotted lines represent 95% confidence limits.
Figure 3.8: Threshold Electrotonus Recordings Comparing the Normal Control Group With The Whole MND Group (Ulnar Nerve - Above Elbow Stimulation)

**A:** Superimposed threshold electrotonus waveforms from 17 normal control subjects. Responses recorded from ADM following stimulation of the ulnar nerve above the elbow. Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. **B:** Superimposed threshold electrotonus waveforms from 31 patients with MND. Responses from ADM following stimulation of the ulnar nerve above the elbow. **C:** Mean normal control threshold electrotonus waveform comparing MND (thin line) and normal control (thick line) groups following above elbow stimulation of the ulnar nerve.
Figure 3.9: The Range of Responses at 10-20 ms Delay Comparing The Whole MND and The Normal Control Groups (Ulnar Nerve - Above Elbow Stimulation)

Percentage threshold reduction at 10 - 20 ms delay comparing recordings from 31 patients with MND (right) and 17 normal control subjects (left). The mean percentage threshold reduction is represented by the solid horizontal lines.

The dotted lines represent 95% confidence limits.
Figure 3.10: Threshold Electrotonus Recordings From The Whole MND Group - Examples of Other Nerves Tested.

Mean threshold electrotonus recordings in MND patients from nerves other than the standard ulnar nerve protocols (black lines) superimposed on the range of normal control responses from the ulnar nerve, stimulating at the wrist and recording from ADM (grey lines). **A:** Common peroneal nerve - stimulating at the ankle and recording from extensor digitorum brevis (EDB). **B:** Common peroneal nerve - stimulating at the fibular head and recording from tibialis anterior (TA). **C:** Ulnar nerve - stimulating above the elbow and recording from flexor carpi ulnaris (FCU).
3.4.1.2 Ulnar Nerve (Above Elbow - ADM)

In figure 3.12A, the mean above elbow - ADM traces for the neurological control group (dark line) is superimposed upon the normal control group (wrist - ADM) (grey shaded area) and shows that although included within the normal range, the neurological controls show the same 'squashed' appearance for above elbow - ADM recordings as the normal control group (Fig. 3.3). The similarity is further demonstrated in Figure 3.12B which reveals almost identical means for the neurological control and normal controls when comparing above elbow - ADM traces.

3.4.1.3 Other Motor Recordings

Two of the neurological control patients had standard recordings made from the peroneal nerve. The results of their tests are shown in Figure 3.13 and compared to the mean traces from the corresponding sites in the normal control group. The patient with a history of polio has a depolarising response at the upper limit of normal at 10 - 20 ms delay, whereas at the same delay the patient with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) has a trace at the lower limit of normal. The trace in the patient with CIDP is smoother and more angular than that of the patient with polio, as the former recording was made following the adaptation of a modifying programme, which averaged the responses to a greater degree.

However, eight patients in the neurological control group had recordings made from tibialis anterior (TA), stimulating the peroneal nerve at the fibular head. Figure 3.14 compares the fibular head – TA responses in five patients with MND (fig 3.14A), with these eight neurological controls (fig 3.14B) and the means of these two groups compared with the mean normal control recordings stimulating the ulnar nerve at the wrist (fig 3.14C). The responses to depolarisation in all these recordings are almost identical.
Figure 3.11: Threshold Electrotonus Recordings From The Whole Neurological Control Group (Ulnar Nerve - Wrist Stimulation)

A: Superimposed threshold electrotonus waveforms from 64 patients with neurological conditions other than MND. Responses recorded from ADM following stimulation of the ulnar nerve at the wrist. Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. B: Mean threshold electrotonus waveform from the whole MND group +/- one standard deviation.
Figure 3.12: Threshold Electrotonus Recordings From The Whole Neurological Control Group (Ulnar Nerve - Above Elbow Stimulation)

A. Mean responses recorded from ADM following stimulation of the ulnar nerve above the elbow from 64 patients with neurological conditions other than MND (black line), superimposed on all responses from the normal control group, stimulating the ulnar nerve at the wrist (grey lines). Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. B: Mean threshold electrotonus waveform (above elbow - ADM) from the neurological control group (thick line) compared to the mean waveform (above elbow - ADM) from the normal control group (thin line).
3.5 ANALYSIS OF THE SUB-GROUPS TESTED.

In all groups tested by stimulating the ulnar nerve at the wrist and recording from ADM, including MND patients, normal controls and neurological controls, similar mean threshold reductions to depolarising currents at 10 - 20 ms delay were found. However, on further analysis of the data certain differences between the sub-groups became apparent.

3.5.1 The Motor Neurone Disease Sub-groups

As mentioned in section 2.5, the patients in the motor neurone group were further divided into subgroups of the disease, depending on the distribution of upper and lower motor neurone findings and the site. Those patients with both upper and lower motor neurone signs in two or three spinal regions or in the bulbar region and at least two other spinal regions were classified as ‘amyotrophic lateral sclerosis’ or ‘ALS’ and therefore fell into ‘definite’ and ‘probable’ categories of the El Escorial classification (56). Those with purely bulbar features or predominantly bulbar features, with upper or lower motor neurone signs in one other region, were classified as ‘bulbar’. Whereas, those with predominantly lower motor neurone signs were termed ‘progressive muscular atrophy’ or ‘PMA’ and those with predominantly upper motor neurone signs, ‘primary lateral sclerosis’ or ‘PLS’.

By carrying out statistical analysis on the various sub-groups, concentrating on the threshold reduction to depolarising currents at 10 - 20 ms delay, one can see that the means of each sub-group are very similar (Table 3.1) and the differences between these means are not statistically significant (t-test).
Figure 3.13: Threshold Electrotonus Recordings From Two Patients in the Neurological Control Group (Peroneal Nerve: Ankle – EDB)

Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. A. Mean response recorded from EDB following stimulation of the peroneal nerve at the ankle from a patient with polio (dark line), superimposed on all responses from the normal control group, stimulating the ulnar nerve at the wrist (grey dots). B. Mean response recorded from EDB following stimulation of the peroneal nerve at the ankle from a patient with chronic inflammatory demyelinating polyneuropathy (CIDP) (dark line), superimposed on all responses from the normal control group, stimulating the ulnar nerve at the wrist (grey dots).
Figure 3.14: Threshold Electrotonus Recordings Comparing the MND Patients and Neurological Controls (Peroneal Nerve: Fibular Head to Tibialis Anterior)

Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. Responses recorded from TA following stimulation of the peroneal nerve at the fibular head. A: Superimposed threshold electrotonus waveforms from 5 MND patients. B: Superimposed threshold electrotonus waveforms from 8 neurological controls. C: Mean ulnar nerve normal control threshold electrotonus waveform (grey line) superimposed upon the mean peroneal nerve (fibular head – TA) electrotonus waveforms from the MND patients (thicker dark line) and neurological controls (thin line).
If however one looks at the range of responses to depolarising currents at 10 - 20 ms, as demonstrated in Figure 3.7, it becomes apparent that the range of the MND group is greater than that of the normal control group. When the subgroups of the MND group are looked at individually in the same format (Fig. 3.15), it becomes clearer that those responses that lie outside the normal range are largely derived from the definite ALS and PMA groups. The responses from the bulbar and PLS groups fall within the normal range. This can be statistically demonstrated if the analysis of the variance of the sub-groups is studied (f - test). A significantly increased variance is found in both the ‘ALS’ and ‘PMA’ groups, compared to the normal controls, neurological controls, bulbar and ‘PLS’ groups. P values for all comparisons between the ‘ALS’ or ‘PMA’ groups against the other sub-groups reach highly significant levels (e.g. ALS vs normal controls: \( p = 3.4 \times 10^{-7} \)) (Table 3.1), whereas those comparing the ‘ALS’ against the ‘PMA’ subgroups or any of the other subgroups against each other are not significant.

Of note is that the whole MND group has a highly significant variance when compared to the normal controls (\( p = 4.7 \times 10^{-7} \)). Also, when the one patient in the ALS group with a threshold reduction of 9.5 at 10 - 20 ms delay was excluded, the analysis of variance between the ‘ALS’ and normal control groups is still highly significant (\( p = 0.0001 \)) and thus this one patient is not skewing the data. It therefore appears that the classical ‘MND abnormalities’, previously found with threshold electrotonus are only apparent in subgroups of patients with lower motor neurone features in spinal segments(i.e. ‘ALS’ and ‘PMA’ groups) and that the remaining groups (i.e. ‘Bulbar’ and ‘PMA’) produce patterns that are very similar to normal controls.
Figure 3.15: The Range of Responses at 10-20 ms Delay Comparing MND Subgroups and Normal Controls (Ulnar Nerve: Wrist - ADM)

Percentage threshold reduction at 10 - 20 ms delay comparing recordings from patients with MND divided into 'definite ALS', 'Bulbar', 'PMA' and 'PLS' subgroups. The mean percentage threshold reduction is represented by the solid horizontal lines. Patients in the 'Definite ALS' and 'PMA' groups show a variability greater than the normal range.
3.5.2 Familial MND

One patient with MND was positive for the SOD-1 mutation. He had signs, which would otherwise have included him in the 'Definite ALS' group, according to the El Escorial criteria. His threshold electrotonus recordings taken on three separate occasions, at three monthly intervals, are shown in Figure 3.16 and are compared to the normal control range. The responses to depolarisation on each occasion are almost identical, despite continued clinical deterioration, and lie at the upper limit of the normal range.

3.5.3 The Neurological Control Sub-groups

The neurological controls can be sub-divided into patients with conditions affecting the upper and lower motor neurones. The lower motor neurone sub-group can further be divided into patients in whom the primary pathology is in the anterior horn cell i.e. old polio, post-polio syndrome and spinal muscular atrophy and those in whom the primary pathology is distal to the anterior horn cell. Subjects with benign fasciculation are considered separately, as the condition is considered to be found in otherwise normal subjects. Finally, the findings from the patient with neuromyotonia will be considered separately.
<table>
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<th>Group</th>
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<th>Variance</th>
<th>P Values for comparison of variance</th>
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<td>NC</td>
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<td>68.4</td>
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</tr>
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</tr>
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<tr>
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<td>68.1</td>
<td>18.7</td>
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</tr>
</tbody>
</table>

**Table 3.1: Comparison of Means and Variance of Threshold Reduction on Depolarisation in the Subgroups of Patients with Motor Neurone Disease and the Normal Control and Patient Control Subjects.**

The mean threshold reduction at 10 – 20ms delay is similar in all patient subgroups and controls. However, the variance is far greater in the ALS and PMA groups compared to any other group tested. [n/a = not applicable].
Figure 3.16: Three Threshold Electrotonus Recordings From A Patient Positive for the SOD-1 Mutation Compared to Normal Controls (Ulnar Nerve: Wrist – ADM)

Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. Three recordings made at three monthly intervals from an SOD-1 positive patient (dark lines), superimposed on the normal control recordings (grey dots).
3.5.3.1 Upper Motor Neurone Conditions

Subjects considered in this subgroup included patients with MS (48), Parkinson’s disease (1), multiple systems atrophy (1), cervical myelopathy (1), generalised dystonia (1) and CVA (1). The patient with Friedrich’s ataxia has been included in both the upper and lower motor neurone groups. A fuller analysis of the MS group can be found in Chapter 8.

All patients in the upper motor neurone subgroup exhibited a similar range of threshold electrotonus waveforms (Fig. 3.17A/B) and excitability changes to depolarisation within the normal range between 10 - 20 ms delay (Fig 3.18). The mean threshold electrotonus waveform of the UMN group is almost identical to that of the normal control group, with only a minimal difference seen in the response to hyperpolarisation (Fig.3.17C and 3.18 solid horizontal line).

3.5.3.2 Lower Motor Neurone Conditions and Diseases Involving Muscle

Subjects considered in this subgroup excluded those with anterior horn cell pathology, those with benign fasciculations and the patient with neuromyotonia but included patients with multifocal motor neuropathy (2), ulnar neuropathy (4), neuralgic amyotrophy (3), CIDP (6), peripheral sensory neuropathy (4), acute nerve injury (1), myopathy (1), late - onset myotonia (1), and Friedrich’s ataxia (1).

All subjects fell within the normal range between 10 - 20 ms delay for recordings from wrist - ADM (Fig 3.18). The lower motor neurone subgroup produced a similar range of threshold electrotonus waveforms when compared to the normal control group (Fig. 3.19A/B). The mean threshold electrotonus waveform of the LMN group is almost identical to that of the normal control group, apart from a slight difference in the recovery period following hyperpolarisation (Fig 3.19C).
Figure 3.17: Threshold Electrotonus Recordings Comparing The Upper Motor Neurone Neurological Control Subgroup with the Normal Control Group (Ulnar Nerve – Wrist - ADM)

Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. A: Superimposed threshold electrotonus waveforms from patients with UMN conditions. B: Superimposed threshold electrotonus waveforms from the normal control group. C: Mean normal control threshold electrotonus waveform comparing patients with UMN conditions (thin line) and normal control (thick line) subjects. (UMN: upper motor neurone)
Figure 3.18: The Range of Responses at 10 -20 ms Delay
Comparing Neurological Control Subgroups and Normal Controls (Ulnar Nerve: Wrist - ADM)

Percentage threshold reduction at 10 - 20 ms delay comparing recordings from patients with neurological conditions other than MND divided into 'UMN', 'LMN', 'Polio and SMA' and 'Benign fasciculations' subgroups. The mean percentage threshold reduction is represented by the solid horizontal lines. (‘UMN’: upper motor neurone; ‘LMN’: lower motor neurone; ‘SMA’: spinal muscular atrophy).
3.5.3.3 Anterior Horn Cell Conditions and Benign Fasciculations

Subjects considered in the ‘anterior horn cell’ subgroup included 15 patients with a history of polio and 7 with spinal muscular atrophy. 11 subjects with benign fasciculations were studied.

Subjects in the anterior horn cell group exhibited excitability changes within the normal range except one patient who had a threshold reduction at 10 - 20 ms above the normal range. This patient had a history of polio and a recent onset lower motor neurone, PMA-like condition which was diagnosed as ‘post - polio syndrome’. All subjects in the benign fasciculation subgroups exhibited excitability changes to depolarisation within the normal range between 10 - 20 ms delay (Fig 3.18). Both groups had a similar range of threshold electrotonus waveforms (Figs. 3.20A/B and 3.21A/B). The means of both groups when compared to the mean normal control group were almost identical, especially the benign fasciculation group (Figs. 3.20C and 3.21C).

3.5.3.4 Neuromyotonia

Acquired neuromyotonia, or Isaac's syndrome, is being considered separately since it is caused by autoantibodies directed against motor nerve, voltage -gated potassium channels (140). The syndrome consists of hyperexcitability of peripheral motor nerves leading to myokymia, weakness, muscle stiffness present at rest which increases with exertion and delayed relaxation after voluntary contraction. All symptoms respond well to treatment with phenytoin or carbamazepine (141) and may be temporarily abolished with plasma exchange (142).

Electromyography reveals characteristic spontaneous discharges firing rhythmically and continuously in affected muscle groups, some representing motor units, others single fibre discharges. The motor activity continues during sleep and through anaesthesia. Following
Figure 3.19: Threshold Electrotonus Recordings Comparing The Lower Motor Neurone Neurological Control Subgroups with the Normal Control Group (Ulnar Nerve – Wrist - ADM)

Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. A: Superimposed threshold electrotonus waveforms from patients with LMN conditions. B: Superimposed threshold electrotonus waveforms from the normal control group. C: Mean normal control threshold electrotonus waveform comparing patients with LMN conditions (thin line) and normal control (thick line) subjectse. ('LMN': lower motor neurone)
Figure 3.20: Threshold Electrotonus Recordings Comparing Patients with Anterior Horn Cell Conditions with the Normal Control Group (Ulnar Nerve – Wrist – ADM)

Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. A: Superimposed threshold electrotonus waveforms from patients with anterior horn cell conditions (polio and SMA). B: Superimposed threshold electrotonus waveforms from the normal control group. C: Mean normal control threshold electrotonus waveform comparing patients with anterior horn cell conditions (polio and SMA) (thin line) and normal control (thick line) subjects. ('SMA': spinal muscular atrophy)
Figure 3.21: Threshold Electrotonus Recordings Comparing Subjects with Benign Fasciculations with the Normal Control Group (Ulnar Nerve – Wrist - ADM)

Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. **A:** Superimposed threshold electrotonus waveforms from subjects with benign fasciculations. **B:** Superimposed threshold electrotonus waveforms from the normal control group. **C:** Mean normal control threshold electrotonus waveform comparing subjects with benign fasciculations (thin line) and normal control (thick line) subjects.
voluntary activation or motor nerve stimulus, similar repetitive afterdischarges result (143).

The most characteristic findings when recording threshold electrotonus from the patient with neuromyotonia, who was found to have potassium channel antibodies, were an unusual threshold electrotonus waveform and an oscillating threshold.

Figure 3.22A compares the recording made from this patient with a recording made from an ischaemic nerve in a normal subject and shows very similar 'squashing' of the threshold electrotonus responses to both depolarisation and hyperpolarisation. If the potassium channel dysfunction, caused by the presence of antibodies, was to be responsible for the abnormality in the threshold electrotonus recording, a waveform similar to a Type 1 or Type 2 response would be expected. However, in view of the appearance of the threshold electrotonus waveform, it is possible that ischaemia caused by sustained muscular contraction was responsible.

The oscillation of the threshold in the patient with neuromyotonia is demonstrated in Figure 3.23A where the threshold current is seen to oscillate similarly in response to the test pulse and during the depolarising and hyperpolarising currents. This is compared to a normal control responses which show the classical divergence that is expected (Fig. 3.23B).

In addition, it was found that there was a prolonged increase in excitability after each stimulus which was response - dependent, rather than stimulus - dependent. As a result a new stimulation protocol, with varying interstimulus intervals and varying stimulus intensities, was written to assess whether further information could be gleaned.

Figure 3.24, illustrates the protocol (Fig. 3.24A) and the resulting responses (Fig. 3.24B) in the patient with neuromyotonia. When the response differences at 250 ms interstimulus delay are related to the
stimulus (Fig. 3.24C), a marked divergence is apparent between the responses to the decreasing compared to the increasing stimuli. This finding is not seen when using the same protocol in a normal control subject (Fig. 3.25), where instead the responses to increasing and decreasing stimuli are identical for a given stimulus and interstimulus interval. Moreover, it is apparent that this divergence is still present when the interstimulus interval is 1000ms (Fig. 3.24D) in the patient with neuromyotonia, but not in the normal control subject (Fig. 3.25D). This phenomenon may be related to the prolonged repetitive firing following each stimulus.

Thus in the study of this patient with neuromyotonia, the threshold electrotonus findings presented a pattern indistinguishable from ischaemia which may have been caused by sustained muscular contraction. If this is the case the ischaemia prevented the possibility of elucidating whether the abnormalities caused by the potassium channel antibodies could be picked up using the technique. Slow oscillations in the threshold were also seen, which were probably caused by prolonged repetitive firing and hyperexcitability following each impulse.

Recently, further studies of patients with neuromyotonia in Japan using this technique have produced similar recordings in the absence of ischaemia, as measured by oxygen saturation laser dopplers placed over the stimulation and recording sites (Arimura, personal communication). Recordings of partial pressure of oxygen (pO₂) were within the normal range.
Figure 3.22: Threshold Electrotonus Recordings Comparing a Patient with Neuromyotonia and an Ischaemic Nerve in a Normal Control Subject (Ulnar Nerve – Wrist – ADM)

Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. **A:** Threshold electrotonus waveforms in response to +/-20% and +/-40% polarising currents from a patient with neuromyotonia. **B:** Threshold electrotonus waveforms in response to +/-40% polarising currents from a nerve made ischaemic in a normal control subject.
Figure 3.23: Threshold Current Response to the Threshold Electrotonus Protocol Comparing a Patient with Neuromyotonia and a Normal Control Subject. (Ulnar Nerve – Wrist – ADM)

Threshold current response to the test pulse (dark line, middle), +40% depolarising currents (dark line, upper) and to -40% hyperpolarising currents (grey line). A: Oscillating threshold current in response to the threshold electrotonus protocol in a patient with neuromyotonia. B: Normal divergence of threshold currents in a normal control subject.
Figure 3.24: Modified Stimulation Protocol with Varying Interstimulus Intervals and Stimulus Intensities in a Patient with Neuromyotonia (Ulnar Nerve – Wrist - ADM)

A: Five alternating stimulus intensities used in this modified protocol. B: Response to each stimulus (colour matched). C: Response (as a percentage of the maximum) at 250 ms in response to the stimulus increasing (green) and decreasing (red). D: Response difference (as percentage maximum response) at 250 ms (black), 500 ms (red) and 1000ms (blue).
Figure 3.26: Modified Stimulation Protocol with Varying Interstimulus Intervals and Stimulus Intensities in a Normal Control Subject (Ulnar Nerve - Wrist - ADM)

A: Five alternating stimulus intensities used in this modified protocol. B: Response to each stimulus (colour matched). C: Response (as a percentage of the maximum) at 250 ms in response to the stimulus increasing (green) and decreasing (red). D: Response difference (as percentage maximum response) at 250 ms (black), 500 ms (red) and 1000 ms (blue).
3.6 DISCUSSION

Seventy patients with MND were tested with threshold electrotonus as a means of assessing the reproducibility of the previous study and assessing the technique's use as a diagnostic tool in the disease. These recordings were compared with 35 normal controls and 64 patients with other neurological conditions.

All neurological controls produced threshold electrotonus recordings within the normal range, apart from the one patient with neuromyotonia, who exhibited the effects of post-stimulation afterdischarges and possibly ischaemia on the threshold electrotonus recordings.

Although the mean age of the normal and the MND groups differed, the previous study (21) revealed no effect of age on the threshold electrotonus waveform and the current study confirmed these findings when comparing the older subjects with the younger ones.

Unlike the initial study, only 28% of the patients with MND exhibited an abnormality (18 patients - "type 1" and 2 patients - "type 2") when stimulating the ulnar nerve at the wrist. Recordings within the normal range were found in the majority, including the patient who was positive for the SOD-1 mutation. Moreover, the abnormalities were only found in MND patients with lower motor neurone signs in the limbs i.e. the 'ALS' and 'PMA' subgroups. Recordings taken from both wrists were identical in all cases, except one patient discussed in chapter 5. Recordings from nerves other than the ulnar nerve were limited in number but all these were within the normal range.

Thus, threshold electrotonus appears to be a specific test for subgroups of MND affecting the lower motor neurone, however it is not sufficiently sensitive to be used routinely as a diagnostic test. The proposed ionic imbalance (21) does not appear to be uniformly present in patients with
MND. Even in those patients with rapidly progressive disease and in the cases where the abnormalities were found, there did not appear to be a correlation with the disease progress.
4. SINGLE MOTOR UNIT RECORDINGS

4.1 INTRODUCTION

Bergmans (144) pioneered the use of threshold measurements for the study of human motor axons. He found that a single motor unit could often be activated selectively by surface stimulation. His method was somewhat difficult and involved first finding a single unit that could be activated selectively with surface electrodes, and then determining its threshold manually, as the voltage which would excite the unit in three out of five stimuli. With the advent of Raymond's 'threshold hunter' (145), a feedback circuit which adjusted stimulus duration automatically to keep the probability of exciting a frog axon close to 50%, a solution to the difficulty of threshold determination was found. The subsequent development of threshold electrotonus provided an ideal alternative for studying human nerves *in vivo*.

In normal subjects, isolating a single unit is difficult and time-consuming and it became apparent that one could define 'threshold' for a compound muscle action potential (CMAP) which behaved, in most cases in exactly the same way as the threshold for a single unit (19). Therefore the use of a CMAP set to a specified fraction of the maximal response became the preferred method when using threshold electrotonus, especially in normal subjects or patients with normal muscle bulk.

However, when studying patients with MND, only a proportion of the motor units are abnormal and it was felt that by recording excitability changes in single units, more information might be gleaned. In patients
with wasted muscles secondary to axonal loss, the isolation of a single unit by surface stimulation is relatively easy.

4.2 METHOD

A separate computer protocol was written for the study of single motor units, however the basic method of recording threshold electrotonus remained the same as described in section 2.4. Electrodes were placed as previously, and the test stimulus alone was introduced and increased until a single unit was elicited. The peak height was adjusted to track this single unit, such that there was an all or nothing response and no other unit was fired. The standard threshold electrotonus protocol was then started with 100ms polarising currents set to +40% and -40% of the previous test pulse superimposed on the 1ms test pulse, with the interstimulus interval being increased as before.

In view of the difficulty in isolating single units in normal subjects, this protocol was only carried out on patients with MND. As mentioned previously, tests other than the conventional recording of CMAPs from the ulnar nerve at the wrist were only carried out on patients who were followed up longitudinally. Of those, it was only possible to isolate a single unit in patients with sufficiently wasted muscles and thus only patients in the ‘ALS’ and ‘PMA’ groups were included. 14 patients had single units successfully isolated (8 with ALS; 6 with PMA).

It was also hoped to be able to follow a particular single unit over time by carefully recording the exact site of electrode placement (with measurements from fixed points and photographs) and noting the exact window parameters. However, it proved to be impossible to locate the same unit on subsequent occasions, which is not surprising as MND is a progressive disease in which axons are constantly dying or sprouting to reinnervate denervated muscle. Nevertheless, repeated single unit
recordings from different units were made in 7 of the 14 patients (3 with ALS; 4 with PMA).

4.3 RESULTS

Figure 4.1A shows the superimposed single unit traces from the patients in the ALS group. Two patients (25%) exhibited increased excitability to depolarisation between 10 - 20 ms delay whereas the remaining patients had waveforms within the normal range. Moreover, the mean trace of responses to depolarisation from this group was flatter than the mean normal control trace, recording from CMAPs (Fig. 4.1B).

In the PMA group, three patients (50%) exhibited heightened excitability to depolarisation between 10 - 20 ms delay (Fig. 4.2A). The mean trace of responses to depolarisation from this group followed a typical Type 1 pattern when compared to the mean normal control group, recording from CMAPs (Fig 4.2B)

Despite the failure to isolate the same motor unit longitudinally in individual patients, Figure 4.3 shows that the threshold reduction to depolarisation at 10 - 20 ms delay for single unit recordings, was very similar in six of the patients tested on different occasions. The subject depicted by the open circles represents a patient with MND before and after he started on carbamazepine. His details and traces are expanded upon in Section 6.3.

In eleven patients both the CMAP and single unit recordings were within the normal range and almost identical to each other. Examples of these recordings are shown in Figure 4.4. In three patients, abnormal (Type 1) CMAP traces were mapped almost exactly by the single unit traces (Fig. 4.5). It was only in one patient that abnormal (Type 1) single unit traces were obtained in the presence of a normal CMAP trace (Fig. 4.6).
Figure 4.1: Single Motor Unit Threshold Electrotonus Traces From Patients in the ‘Definite ALS’ Subgroup (Ulnar Nerve – Wrist - ADM)

Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. A: Superimposed single unit threshold electrotonus waveforms from 8 patients in the ‘Definite ALS’ subgroup. B: Mean compound muscle action potential (CMAP) trace from the normal controls compared with the mean single unit trace from patients in the ‘Definite ALS’ subgroup. (ALS: Amyotrophic lateral sclerosis)
Figure 4.2: Single Motor Unit Threshold Electrotonus Traces From Patients in the PMA Subgroup (Ulnar Nerve – Wrist - ADM)

Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. **A:** Superimposed single unit threshold electrotonus waveforms from 6 patients in the 'PMA' subgroup. **B:** Mean compound muscle action potential (CMAP) trace from the normal controls compared with the mean single unit trace from patients in the 'PMA' subgroup. (PMA: Progressive muscular atrophy)
Figure 4.3: Threshold Reduction Between 10 – 20 ms Delay Comparing Repeated Single Motor Unit Recordings on Separate Occasions From Patients with MND (Ulnar Nerve – Wrist - ADM)

Two single motor unit recordings recorded on separate occasions from three patients in the ‘Definite ALS’ group and four patients in the ‘PMA’ group. The subject depicted by the open circles represents a patient pre- and post- carbamazepine use (see text and Section 6.3).
Figure 4.4: Examples of Normal Single Motor Unit Threshold Electrotonus Traces Compared to Normal CMAP Recordings in the Same Patient (Ulnar Nerve – Wrist - ADM)

Single unit traces (thin lines); CMAP traces (dotted lines). Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. **Case 2:** Two single unit threshold electrotonus waveforms superimposed on three CMAP waveforms from a patient in the ‘PMA’ subgroup. **Case 3:** A single unit threshold electrotonus waveform superimposed on two CMAP waveforms from a patient in the ‘PMA’ subgroup. **Case 5:** A single unit threshold electrotonus waveform superimposed on two CMAP waveforms from a patient in the ‘Definite ALS’ subgroup.
Figure 4.5: Examples of “Type 1” Single Motor Unit Threshold Electrotonus Traces Compared to “Type 1” CMAP Recordings in the Same Patient (Ulnar Nerve – Wrist - ADM)

Single unit traces (thin lines); CMAP traces (dotted or dark lines). Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. Type 1 CMAP and single unit recordings from: Case 4: a patient in the ‘Definite ALS’ subgroup. Case 6: a patient in the ‘PMA’ subgroup. Case 8: a patient in the ‘Definite ALS’ subgroup.
Figure 4.6: An Example of “Type 1” Single Motor Unit Threshold Electrotonus Traces Compared to a Normal CMAP Recording in the Same Patient (Ulnar Nerve – Wrist - ADM)

*Single unit traces (dotted lines); CMAP traces (thin lines). Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. Two type 1 single unit recordings made three months apart compared with a normal CMAP recording in a patient in 'Definite ALS' subgroup (case 1).*
4.4 DISCUSSION

The technique of isolating single motor units by surface stimulation is a difficult and time-consuming process. With the use of the adapted threshold electrotonus protocol, the process has been made simple, but is only truly feasible when recording from patients with sufficient axonal degeneration and muscle wasting.

Recording threshold electrotonus from single units revealed abnormalities in only one patient that were not apparent when recording from CMAPs, whereas in the remaining 13 patients the CMAP and single unit recordings were almost identical.

When studying the normal, as well as abnormal units, during recordings from CMAPs, the features of the abnormal units may be averaged out. However, the recording of single motor units using threshold electrotonus, only allows the threshold to be tracked from the first unit that can be isolated, which may be a normal unit. As was apparent from the results, the pick-up rate of abnormal units was low.

In thirteen out of the fourteen patients tested using the single unit protocol, the threshold electrotonus recordings were virtually identical to the CMAP protocol. Changes with time were also similar to longitudinal recordings using the CMAP protocol (see Chap. 5). This therefore concurs with the initial findings of Baker and Bostock (19), and leads to the proposal that rarely is any useful additional information gleaned from the more complex study of single motor units.
5. RESULTS - LONGITUDINAL RECORDING

MOTOR NEURONE DISEASE STUDY

5.1 INTRODUCTION

In order to assess whether the proposed ion channel dysfunction, which produced either a Type 1 or Type 2 abnormality in patients with MND (21), represented a progression with the course of the disease, 37 patients were followed up. They had repeated threshold electrotonus recordings at 2 - 4 monthly intervals for as long as possible over the 2 year period of the research.

In the initial clinical study of threshold electrotonus, only single recordings were made on the patients and controls (21). Therefore, the finding of Type 1 or Type 2 abnormalities in patients with MND, could not put the progression of the proposed ion channel dysfunction into the context of their disease progression. From this study, it was hypothesised that a Type 1 abnormality was an early manifestation of the disease and represented fast potassium channel dysfunction as indicated by further experiments involving rat axon and a computer model of human axon (21). A Type 2 abnormality, which indicated dysfunction of both fast and slow potassium channels (21), was therefore hypothesised to represent a subsequent progression

5.2 METHODS

The standard threshold electrotonus protocol was used for all follow up recordings. On each occasion a recording was made from ADM stimulating the ulnar nerve at the wrist, to provide a standard
comparison both between patients and in the individual patient with time, and, in addition, recordings were often made from other sites.

37 patients with MND were followed up. Of these 27 provided comparable recordings. Most recording were made in the patients' homes using the portable system, in order to save them having to travel to the hospital at such regular intervals. The breakdown of the numbers in each subgroup of MND in this arm of the study was as follows: 22 with definite ALS; 7 in the bulbar group; 4 with PMA; 3 with PLS and 1 with familial ALS, positive for the SOD-1 gene.

5.3 RESULTS

Of the 37 patients with MND who were followed up, 6 patients exhibited Type 1 waveforms on initial testing and 1 patient exhibited a Type 2 waveform in one hand. None of the patients with normal waveforms progressed over the two-year period to produce a Type 1 or 2 response, as was hypothesised, despite clinical progression of their disease. Those with Type 1 responses did not progress to Type 2 patterns, again, despite progression of their disease. However, one patient with a Type 1 pattern on initial testing, normalised her threshold electrotonus trace with time (see Fig. 5.2).

The individual with the Type 2 response in one hand had a striking asymmetry clinically, with marked wasting of the left arm and a relatively normal looking right arm. Standard neurophysiological tests confirmed acute and chronic partial denervation in all four limbs, but were most pronounced in the left hand. There were no abnormalities in the nerve conduction studies and no evidence of conduction block. Interestingly, he progressed from a Type 2 to a Type 1 waveform over a 7-month period in the left hand, but the responses from the clinically unaffected hand remained almost identical (Fig 5.1).
Figure 5.1: Threshold Electrotonus Waveforms from a Patient with Clinically Asymmetrical MND

This 47 year old gentleman had severe wasting and weakness of the left hand and, to a lesser extent, left arm and brisk reflexes in all limbs. Conventional electrophysiology revealed chronic partial denervation in four limbs which was most marked in the left hand and arm. **A:** The threshold electrotonus recordings, taken seven months apart from the right (clinically unaffected) hand were almost identical. **B:** The first recording from the left (affected) hand revealed a "Type 2" abnormality, with a marked fall in threshold reduction early in the response to +40% depolarisation. A second recording, made seven months later from the same hand revealed a "Type 1" abnormality with a marked increase in threshold reduction in response to +40% depolarisation.
The normal and neurological controls that were tested on multiple occasions produced waveforms that were almost identical on each occasion, with changes in threshold reduction, between 10 – 20 ms delay, that were less than 2%. There was however, one normal control subject who exhibited an increase in threshold reduction at this delay of 5.5% and therefore only changes greater than this amount were deemed significant in the MND group.

The patients with MND were subdivided into those with lower motor neurone (LMN) signs i.e. definite ALS and PMA, and those without i.e. bulbar and PLS. The change in threshold reduction between 10 – 20 ms delay in the latter group was similar to the normal controls, with little variability with time. However, those patients with definite ALS and PMA showed far greater variability. There was no clearcut pattern. Some patients showed little change, whereas others developed marked increases or decreases in threshold reduction with time. Interestingly, those patients that showed the greatest increases, were all patients with definite ALS, who had rapidly progressive disease and who died during the course of the study. However, all their traces fell within the “normal” range. Two of the patients who showed a marked decrease, were those that produced a flattening of their threshold electrotonus waveform (see Fig 5.3). A further subject that showed a marked decrease, was the patient whose trace progressed from a Type 1 to normal pattern. The patient with the asymmetrical recordings is not represented in Figure 5.2.

A new pattern of abnormality was revealed by this study, not previously recorded in previous studies. A few patients demonstrated a flattening of the early part of the trace over time. (Fig 5.3)
5.4 DISCUSSION

Serial recordings in patients with MND and normal controls, showed that the majority of subjects exhibited little change with time. However, patients with definite ALS and PMA exhibited more variability than other groups tested.

All the patients in the normal range, remained within the normal range despite marked progression of their disease at various rates, assessed clinically and using the Appel rating scale (137). Two patients, however showed a very marked increase in their threshold reduction at 10 – 20 ms delay, between the first and last recording, and a further two patients showed a significant increase compared to the normal control group (Fig. 5.2). These patients all had rapidly progressive, definite ALS and died during the course of the study. Despite the failure to confirm the hypothesis that patients with MND progress from a Type 1 to Type 2 pattern, these four patients add weight to the theory that an alteration of membrane properties occurs, and is greater, in patients with a more rapidly progressive disease. In the context of the current findings, this, however, is likely to be a secondary phenomenon and not a feature of the primary pathology.

There were three other exceptions to the general rule. First, the one patient with a strikingly asymmetrical and slowly progressive disease process, who initially exhibited a Type 2 response in the affected hand, which then converted to a Type 1 response. Secondly, the patient who progressed from a Type 1 abnormality to a normal recording and thirdly, there were 3 patients who developed flattening of the early part of the response to depolarisation.

In the first case, the presence of a Type 2 abnormality did not represent a rapidly progressive disease state and in fact this gentleman had a very slowly progressive disease, which was corroborated by an increase of only 4 points in his Appel score over a 2 year period. The loss of the
Type 2 response may have indicated the death of the unstable motor units, which were replaced by further abnormal motor units resulting in the Type 1 pattern subsequently found, rather than to the changing membrane properties within the same motor unit. A similar explanation can be extended to the patient who normalised her previous Type 1 trace. The loss of the Type 1 response may have indicated the death of unstable motor units, with normal units being recorded at the subsequent test.

The newly reported flattening of the response to depolarisation may represent a stage in the progression towards a Type 2 response, however follow up of these patients showed no further deterioration of the response which appeared to plateau in the flattened state. The three patients also had very different disease patterns. One had bulbar palsy with no clinical or electrical involvement of the hand that was tested, one had rapidly progressive generalised ALS and the third had slowly progressive limb-only ALS. The physiological basis for the flattening of the responses is not known. Data from the previous rat and computer models did not produce this pattern of response when manipulating fast and slow potassium channels. It is possible that this finding may represent progressive partial loss of both fast and slow potassium channels occurring together, but not sufficient to produce the classical Type 2 response, but this would require further studies to investigate the cause.

Therefore, the longitudinal study of threshold electrotonus to ascertain whether there was a progression from Type 1 to Type 2 abnormalities in patients with MND was somewhat marred by the finding of a reduced sensitivity of the technique in MND, compared to the initial study (21). A subsequent hypothesis that patients exhibiting a 'normal' threshold electrotonus waveform may progress to a Type 1 abnormality was therefore proposed, however, this too did not prove to be the case.
There did, however, seem to be some positive correlation between a marked increase in threshold reduction with time and a more rapidly progressive disease process. In addition, it was shown that patients with definite ALS and PMA exhibit greater variability of their recordings with time, than was seen in the bulbar, PLS, normal and neurological control groups.
Patients with MND were divided into two groups – those with lower motor neurone signs ('definite ALS' and 'PMA') and those without ('bulbar' and 'PLS'). The threshold reductions at the first and last recording for each subject are represented. All repeat recordings occurred more than six months after the first ones.
Figure 6.3: Flattening of the Threshold Electrotonus Waveforms with Time in Three Patients with MND

Three patients exhibited flattening in their responses to depolarisation with time, losing the initial 'hump' prior to accommodation of the response.
6. THE EFFECTS OF MEDICATION ON THRESHOLD ELECTROTONUS

6.1 INTRODUCTION

Bostock et al's initial clinical study (21) suggested that dysfunctional potassium channels, and therefore an imbalance in the sodium-potassium ratio, might underlie the neuronal damage in MND. We therefore sought to investigate whether drugs that have an effect on sodium channels might redress the proposed imbalance and restore some degree of membrane stability and thus, potentially alter threshold electrotonus recordings and possibly even the course of the disease.

An obvious agent to concentrate on was riluzole, which during the course of this study was under trial and subsequently prescribed on a compassionate named-patient basis for MND. Initially, in addition, it was planned to study the effect of the licensed membrane-stabilising agents, carbamazepine or phenytoin. These drugs are in regular use for epilepsy but had not been used in motor neurone disease. The anti-epileptic medication was only to be given to patients who were not taking riluzole and with the permission of the consultant in charge of the individual patients.

6.2 RILUZOLE

6.2.1 Introduction

Riluzole belongs to the benzothiazole family of compounds and acts on at least four pre-synaptic and post-synaptic processes. It has not been
determined which of these actions account for the neuroprotective properties of the drug.

There have been numerous theories about the pathogenesis of MND and recent studies have focused attention on glutamate excitotoxicity as a possible mechanism (63; 146). Excitotoxicity involves a series of pathophysiological events. High levels of glutamate in the synaptic cleft cause excessive stimulation of the post synaptic cell, activating N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA) / kainate receptors. These receptors regulate ion channels, which on activation cause the entry of sodium and calcium ions into the cell. The resultant depolarisation activates voltage-dependent sodium and calcium channels, causing further depolarisation and disturbance of ionic homeostasis. Calcium-activated lytic enzymes are triggered, resulting in cell lysis. The cytoplasmic glutamate is released causing an amplificatory loop of excitotoxicity.

Increased glutamate levels have been found in the cerebrospinal fluid of patients with MND (70). It has also been shown that rat motor neurones die when incubated with inhibitors of glutamate uptake. The rationale for the use of riluzole in MND was provided by the finding that neuroprotection is produced by drugs that modify this process.

Riluzole modulates glutaminergic transmission by presynaptic inhibition of glutamate release and postsynaptic interference with the effects of excitatory amino acids.

Riluzole is also known to activate a G-protein transduction process, bypassing the ligand-receptor binding which is normally required for activation. G-proteins are cell membrane proteins, which are normally activated by the binding of a ligand to its receptor which subsequently leads to activation of specific enzymes necessary to generate second messengers within the cell. It has been proposed therefore that riluzole
may indirectly modify the activity of excitatory amino acid receptors through this process.

Of particular interest to the current study, is the additional action of riluzole on voltage-dependent sodium channels. It causes inactivation of these channels pre- and postsynaptically, by stabilising their inactivated state (147).

Following the publication of a prospective, randomised, double-blind placebo-controlled trial of riluzole in 959 patients with MND (65), riluzole has now become licensed in the UK. The trial found a significant increase in survival compared to the placebo and the survival benefits were maintained over 18 months in patients with bulbar-onset and limb-onset MND. A further conclusion from this trial was that a dose of 100 mg per day provided optimal benefit and was the recommended dose. This was the dose that all patients, who were on the drug, were taking when tested with threshold electrotonus.

During the period of the study riluzole was available on a compassionate, named-patient basis. Therefore, only patients prescribed the drug by the consultant neurologist in charge of their care were able to take it and thus the numbers of patients recruited into this arm of the study were limited by individual consultants' preferences.

6.2.2 Subjects

Out of the 37 patients followed up regularly, 12 patients were taking riluzole. 3 additional patients had taken it but 2 were unable to tolerate it and the third patient died prior to having a threshold electrotonus recording while on the drug. Of the 12 patients taking riluzole, 10 were seen and had threshold electrotonus tests both prior to and following taking the drug and could therefore could provide data on the effect of riluzole on their disease as assessed by threshold electrotonus.
Standard threshold electrotonus recordings were made on all patients taking riluzole.

6.2.3 Results

As all patients had recordings made from ADM, stimulating the ulnar nerve at the wrist on each occasion, the analysis of the effects of riluzole on threshold electrotonus was made using these recordings.

Figure 6.1 shows the superimposed traces from the patients prior to taking riluzole (Fig. 6.1A), the superimposed traces from the same subjects once taking riluzole (Fig. 6.1B). A comparison of the means of the two groups (Fig. 6.1C) show that following the use of riluzole there was a slight increase in excitability to depolarisation.

The threshold reduction at 10 - 20 ms delay is demonstrated in Figure 6.2 in each patient that was tested pre- and post-riluzole. The majority of patients showed minimal change in threshold reduction following the use of riluzole. One patient developed a particularly large increase in threshold reduction, or increased excitability, but his threshold electrotonus trace remained in the normal range. He was a patient with definite ALS with a rapidly progressive disease process and died during the course of the study.

6.2.4 Discussion

The hypothesis that the effect of riluzole on sodium channels may result in stability of the axonal membrane and thus 'improve' the threshold electrotonus waveform, was not borne out by our study.

In nine of the ten patients tested before and after riluzole use, the variability of the threshold reduction between 10 – 20 ms with time was similar to that found in normal controls (see Fig.5.2). One patient with rapidly progressive ALS showed a marked increase in threshold reduction with time, despite riluzole use. There were no significant
Figure 6.1: Threshold Electrotonus Recordings Comparing MND Patients Before and After Taking Riluzole (Ulnar Nerve: Wrist - ADM)

Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. **A:** Superimposed threshold electrotonus waveforms from 10 MND patients prior to taking riluzole. **B:** Superimposed threshold electrotonus waveforms from the same 10 MND patients once established on riluzole. **C:** Mean threshold electrotonus waveforms from 10 patients prior to taking riluzole (lower depolarising trace) compared to the mean waveforms from the same 10 patients once established on riluzole (lower depolarising trace).
Figure 6.2: Threshold Reduction Between 10 – 20 ms Delay Comparing Individual MND Patients Before and After Taking Riluzole (Ulnar Nerve – Wrist - ADM)
improvement of threshold electrotonus waveforms as was found by Hoffman et al (148), who reported an improvement in 7 out of 21 patients treated with riluzole.

It therefore appears that whichever effect of riluzole is responsible for the mild neuroprotective effect that has been found in patients with MND, that the action on sodium channels is not a major influence, at least not as assessed by threshold electrotonus.

6.3 MEMBRANE STABILISING MEDICATION

6.3.1 Introduction

Carbamazepine and phenytoin are both known to act as membrane stabilisers via a use- and voltage-dependent blockade of sodium channels (149-151), although the exact relationship between their effect on ionic transmembrane fluxes and their antiepileptic action is less certain.

The rationale for their trial in patients with MND arose from the initial finding of the ion channel abnormality found in the first clinical study (21). It was hypothesised that this dysfunction might account for the fasciculations and the fatal depolarisation of the motor neurones and therefore drugs, which stabilise the membrane through sodium channel blockade, may redress the imbalance and may improve or even reverse the process.

Both carbamazepine and phenytoin are well tolerated at concentrations known to reduce axonal sodium conductance. Furthermore, these drugs have been found to protect CNS axons against the damaging effects of anoxic depolarisation at concentrations lower than those employed to treat epilepsy or required to affect action potential generation in vitro (152). The high potency for these drugs in protecting axons from anoxia may be due to their preferential binding to
depolarised axons (151) adding further weight to the hypothesis that they may be of value in preventing fasciculations, type 2 responses and further degeneration in MND.

6.3.2 Subjects

It was planned to assign patients who had abnormal baseline threshold electrotonus recordings and who were not taking riluzole, randomly into two groups, to trial a short course (3 weeks to a month) of either carbamazepine or phenytoin and repeat threshold electrotonus recordings subsequently. Ethics Committee approval was granted. As neither drug is licensed for use in MND, the consultant in charge of the care of each patient involved was approached for permission prior to starting either drug. Ultimately only two patients fulfilled all entrance criteria and were approved for the study. However, the second patient died as a result of a respiratory infection, prior to taking his first dose.

The patient recruited was a 42 year old gentleman, with a two year history of wasting and weakness in his upper limbs and marked fasciculations in his upper and lower limbs. Examination revealed fasciculations in all four limbs and on the trunk. Cranial nerve examination was unremarkable apart from a brisk jaw jerk. There was marked wasting and weakness of his upper limbs, more pronounced distally. The muscle bulk and power were well preserved in the lower limbs. All reflexes were brisk and there was an extensor left plantar response. Sensory examination was normal. EMG examination was consistent with a diagnosis of MND with evidence of active and chronic partial denervation in four limbs. In view of his age and the progress of his disease, it was felt that the possible beneficial effect of riluzole on survival would be negligible and he was therefore an ideal candidate for a trial of membrane stabilising medication. Therefore, carbamazepine was started at a dose of 100mg twice a day and increased after 3 days to 200mg twice a day with no adverse effects.
6.3.3 Results

During the trial period, the patient noticed a marked reduction in his fasciculations and as a result elected to remain on carbamazepine following the trial period.

Two threshold electrotonus recordings from abductor digiti minimi prior to taking carbamazepine and two recordings while on carbamazepine are compared in Figure 6.3B. Fasciculations were evident in ADM pre-carbamazepine but not after taking the drug. The morphology of the threshold electrotonus waveforms are abnormal compared to the normal control range (Fig. 6.3A), however they are a consistent shape at each recording. The responses to depolarisation are virtually identical in the two pre-carbamazepine recordings made 4 months apart. Similarly the responses to depolarisation in the two post-carbamazepine traces are virtually identical, but show a marked difference to those prior to starting the drug. The first post-carbamazepine recording was made after 3 weeks on the drug and the second after 6 months. The threshold reduction to depolarisation between 10 - 20 ms delay fell from an abnormal ('Type 1') level to within the normal range following the use of carbamazepine and was maintained within the normal range with continued use of carbamazepine (fig 6.4).

Over the course of the 11-month follow-up of this patient, there was a marked reduction in fasciculations, which was clinically corroborated, as well as subjectively reported. Otherwise, there was little change in his clinical condition. This was confirmed with repeated scoring using the modified Appel scale (see Section 2.3 and Appendix 1), which showed a one point increase over this period, from a score of 30 to 31.
Figure 6.3: Threshold Electrotonus Waveforms in a Patient Before and After Taking Carbamazepine Compared to the Normal Control Range (Ulnar Nerve - Wrist - ADM)

Threshold changes (top) due to the polarising currents (bottom), both expressed as percentages of the threshold current tested with 1ms test pulses. 

A: Mean normal control trace +/- 1 standard deviation. 

B: Superimposed threshold electrotonus waveforms from the same MND patient (subject BQ) prior to taking carbamazepine (two traces 3 months apart) (dark lines) and once established on carbamazepine (two traces 3 months apart) (thin lines)
Figure 6.4: Threshold Reduction Between 10 – 20 ms Delay in an MND Patient (Subject BQ) Before and After Taking Carbamazepine (Ulnar Nerve – Wrist - ADM)

Two recordings three months apart prior to taking carbamazepine are within the abnormal range at this delay. Two recordings, also three months apart, following the use of carbamazepine are within the normal range at this delay.
6.3.4 Discussion

The results from this arm of the trial amount to a case report of the one patient recruited to take carbamazepine.

The use of carbamazepine in MND has not previously been reported. The marked reduction in fasciculations was not unexpected as similar improvement of fasciculations with carbamazepine have been reported in muscle cramp - fasciculation syndrome (153), neuromyotonia (154) and in the spinal form of Charcot-Marie-Tooth disease (distal spinal muscular atrophy) (155)

The hypothesis that membrane stabilising medication can alter the progression of MND is supported by the marked reduction in fasciculations reported by the patient, the lack of clinical progression, and the improvement in the threshold electrotonus recordings that was maintained with continued use of the drug following the trial period. A larger trial of these well-tolerated drugs, whose pharmacological profiles are well-established, is therefore needed to assess their potential role in the modification of the disease process in MND.
7. TEMPERATURE AND THRESHOLD ELECTROTONUS

7.1 INTRODUCTION

The effects of temperature on standard measures of nerve conduction are well established. The conduction velocity increases almost linearly by 2.4 m/s, or approximately 5 percent per degree centigrade, as the temperature measured near the nerve increases from 29 to 38 °C (156; 157). Similarly the distal latency of the median and ulnar nerves increases by 0.3 ms per degree centigrade on cooling. In addition, as temperature is decreased, the amplitude of nerve and muscle potentials increase. This occurs in squid axon (158) and human nerve (159;160). The increase in amplitude with reduction in temperature appears to be an effect of slowed sodium channel inactivation, as parallel temperature-dependent change occurs in the refractory period (161).

Since Hodgkin and Keynes (162), it has been demonstrated that lowering the temperature causes a marked drop in sodium efflux and thus has an effect on the active process of the sodium pump. Frankenhaeuser and Moore (163) further demonstrated that, in general, channel gating has a $Q_{10}$ of three. In other words, if the temperature is altered by 10°C then the gating changes by a rate of three. However, the effect of temperature on the action of potassium channels has not been previously recorded. The technique of threshold electrotonus provides a unique means of studying the effects of temperature on potassium channels in human nerve in vivo.
7.2 METHOD

A modified threshold electrotonus protocol using 'proportional tracking' was developed for these experiments. The step size was made proportional to the error, which enabled the target response to be reached faster. Thus the responses to +40% and -40% polarising currents were tracked more quickly than normal by advancing the polarising current in 10 ms steps and responses within 10% error were accepted. This protocol produces comparable recordings and allows a test to be completed in about 2 minutes rather than the usual 10 minutes. On occasions responses to +20% and -20% polarising currents were added, extending the test by a further 2 minutes. By using this method a full trace could be obtained within a small temperature range. All recordings made during these experiments utilised this protocol.

In an attempt to perfect the method of altering nerve temperature, three different techniques were employed:

7.2.1 Experiment 1

Skin temperature was altered with the use of a dual heater / air conditioner. The standard threshold electrotonus electrode set-up was used, stimulating the ulnar nerve at the wrist and recording responses from ADM. The arm was positioned within a length of expandable tubing of 23.5 cm diameter and the hand and wrist were positioned outside the tube via a side port in the tube which was adapted to exactly fit the arm just above the anode. This was designed to prevent direct warming or cooling of the nerve and muscle by the airflow. The tubing was connected to the heater/ air conditioner, such that there was free-flow of air through the tube, which escaped at the level of the shoulder. The arm was placed within the centre of the tube with the use of a wedge upon which the elbow rested, avoiding pressure on the ulnar nerve at the elbow. A skin temperature probe was placed on the wrist
next to the stimulating electrode. Temperatures were recorded every 30 seconds. The air conditioner was turned on and the arm was cooled. Repeated threshold electrotonus recordings were made during this period and therefore measurements of latency and threshold reduction were documented throughout.

The airflow was then switched to its heating cycle and the arm was warmed again. Once more threshold electrotonus recordings were made throughout this period.

7.2.2 Experiment 2

In the second method that was utilised, the principle of the water bath was adapted. An elongated container was filled with water at a temperature of 41°C. Similarly, for the cooling protocol, the elongated container was filled with water at a temperature of between 19 and 20.6°C. With the aid of the temperature probe in the water, it was possible to ensure a constant temperature throughout both arms of the experiment.

The electrodes were positioned as in the previous experiments, stimulating the ulnar nerve at the wrist and recording from ADM. The temperature probe was attached to the skin adjacent to the stimulating electrode. The whole arm, with the electrodes and temperature probe in place, was then enclosed in a lightweight, strong polythene bag. The protected arm was then placed in the water bath and repeated threshold electrotonus recordings were made, with skin and water temperature measurements taken every 30 seconds.

7.2.3 Experiment 3

The apparatus for this protocol was very similar to Experiment 1. The aim was to maintain a constant skin temperature at the extremes of warm and cool, which was not possible with the original equipment. The air-conditioner attached to the expandable tubing with a side port was
used. However, rather than alternating the cool and warm cycles, in this experiment a fan heater was connected to the air-conditioner in series. Otherwise the set-up of the electrodes and the remaining apparatus was identical with the hand uninsulated outside the tubing.

Initially, the air-conditioner alone was switched on to cool the arm. Skin temperature was maintained at a plateau by giving brief bursts of heat from the fan heater through the cool air from the air-conditioner, which was not altered.

Similarly, the warming cycle involved turning on the air-conditioner at a reduced level of flow in addition to the fan heater until a warm skin temperature was achieved and this was maintained by giving brief bursts of heat from the fan heater, having turned off the air-conditioner.

As with all previous experiments skin temperature measurements were made every 30 seconds and repeated threshold electrotonus recordings were made throughout.

7.3 SUBJECTS

Six temperature experiments were carried out using the three different protocols.

Experiment 1 was carried out on two normal control subjects (aged 51 and 29 years respectively). Three subjects were tested using the water bath protocol - a normal control subject (51 years), a normal control subject with an abnormal resting threshold electrotonus trace (34 years) and a patient with relapsing-remitting multiple sclerosis (35 years), who gave informed consent. Experiment 3 was carried out on a normal control subject (51 years). The 51 year old subject was the same person in each of the experiments.
7.4 RESULTS

7.4.1 Skin Temperature Change
Resting temperatures ranged between 29.9 to 32.4 °C (mean 30.9°C) in the six subjects. During the cooling protocols, skin temperature ranged between 23.6 and 26.5°C (mean 25.1°C) in the six subjects and rose to between 34.1 and 37.6°C (mean 36.2°C) during the warming protocols.

The rate of change of skin temperature was equivalent for each protocol. It fell by between 0.2 and 0.4°C per minute during the cooling cycles and rose by between 1.1 and 1.3°C per minute during the warming cycles. Only Experiment 3 allowed a constant plateau to be achieved at the extremes of cooling and warming.

7.4.2 Skin Temperature and Latency
There was a close inverse relationship between skin temperature and latency in all subjects. The skin temperature change preceded the latency changes by between 1 and 3 minutes. This relationship is illustrated in Figure 7.1A and B.

7.4.3 Effect of Temperature on Depolarisation
With cooling a dramatic increase in threshold reduction to subthreshold depolarising currents became evident in all subjects, which was maximal between 40 and 70 ms delay (Fig. 7.2A). Warming from cold produced a complete reversal of this effect. Depending on the resting temperature, warming from rest produced little change.

The close correlation between skin temperature and the effect on depolarisation is illustrated in Figure 1B and D, which shows the threshold reduction at 60ms delay.

In addition, a further change was seen in the response to depolarisation at earlier delays, which was somewhat more variable between subjects.
This involved a slight flattening of the trace between 5 and 10 ms delay, at the extreme cold temperatures.

7.4.4 Effects of Temperature on Hyperpolarisation

Cooling reduced the responses to hyperpolarising currents in all subjects and warming increased the responses to hyperpolarising currents.

This response followed a different time course to the changes in latency and skin temperature (Fig 7.1C). In some experiments the response to hyperpolarising currents seemed to correspond to the rate of change of temperature and produced an 'overshoot' which then returned towards the baseline level (Fig 7.1C). This is also illustrated by Figure 7.2B, which comprises two superimposed threshold electrotonus traces taken from a single subject during the same experiment. Despite the actual skin temperatures in the two traces being similar (27.6 and 28.2°C), there is a marked difference between the hyperpolarising responses, with the trace that was taken during the warming cycle producing an increased threshold reduction. Whereas in Figure 7.2A, the two superimposed threshold electrotonus traces, from the same subject and during the same experiment as Figure 7.2B, were taken at skin temperatures that are very disparate (28.2 and 36.2°C) but the hyperpolarising responses are almost identical.

7.4.5 Effects of Temperature on Recovery

Reduction in temperature caused a delayed recovery with accentuation of the threshold reduction in all subjects. Figure 7.2A shows that in two superimposed threshold electrotonus traces taken at a cool (28.2°C) and warm (36.2°C) skin temperature from a single subject during the same experiment, the recovery arms show an overshoot in threshold reduction and a further 60ms delay until the resting level has been achieved.
Figure 7.1: The Effects of Temperature on Latency and Threshold Reduction Following Polarising Currents in a Normal Subject

A: Latency changes (in ms) seen as the skin temperature was decreased then increased. B: Skin temperature changes (in °C) recorded by a probe next to the stimulating electrode. Temperature altered using the protocol in Experiment 1 (see text). C: Threshold reduction (in mV) at 95 ms delay following -40% hyperpolarising currents, in response to changing temperature. D: Threshold reduction (in mV) at 60 ms delay following +40% depolarising currents in response to changing temperature.
7.5 DISCUSSION

Unlike isolated nerve preparations, it is more difficult to control temperature changes *in vivo* and more difficult to gauge actual nerve temperature. However, it has been previously shown that skin temperature measured with a thermistor correlates linearly with subcutaneous and intra-muscular temperature (164;165).

To separate changes due to dynamic and static responses, three types of apparatus were trialled. All three experimental protocols allowed lowering and raising the skin temperature to well below and above normal. Only the apparatus used in Experiment 3, however, allowed a constant plateau to be achieved. Moreover, the three protocols produced an equivalent rate of change of temperature for the cooling and warming cycles.

An inversely proportional relationship exists between skin temperature and latency. Skin temperature changes preceded latency change by just over a minute in most subjects. That a lag does occur indicates that a temperature gradient does exist between the nerve and skin, but without the use of invasive temperature probes this cannot be overcome. Nevertheless, monitoring the latency change is an accurate means of gauging nerve temperature, and we have demonstrated that skin temperature recordings provide a convenient means of monitoring the trends when nerve temperatures are changing, by indicating the direction and rate of change. Moreover, once a plateau has been achieved, the measurement of skin temperature provides an useful means of assessing nerve temperature.
Figure 7.2: The Effects of Temperature on Threshold Electrotonus Waveforms in a Normal Subject

Threshold changes (top) due to polarising currents (bottom), both expressed as percentages of the control threshold tested with 1 ms pulses. A: Two superimposed recordings when the skin temperature measured 28.2°C (extreme of cooling experiment) and 36.2°C (extreme of warming experiment). The response to hyperpolarisation is identical, however there is a marked difference in the response to depolarisation maximal between 40 – 70 ms delay. B: Two superimposed recordings made during the warming and cooling cycles, when the skin temperatures were similar (27.6°C and 28.2°C). The response to depolarisation is similar in each case, however there is a marked difference in the response to hyperpolarisation, maximal between 80 – 100 ms delay.
We also demonstrated an inverse relationship in all our subjects between skin temperature and threshold reduction in response to subthreshold depolarising currents on cooling and then warming to resting levels. This phenomenon was maximal in the later parts of the trace, between 40 – 70 ms delay (Figs 7.1D and 7.2A), which is known to be a function of slow potassium conductance (1). Temperature alters the kinetics of gKs ie how fast it repolarises. On warming the cooled hand the reverse phenomenon occurred. However, warming above resting skin temperatures did not alter the threshold reduction at 60 ms delay any further, but changes continued to occur earlier in the trace.

The effect of temperature on hyperpolarisation is somewhat more complex. Cooling caused a reduction in the responses to hyperpolarising currents. However, warming had a dramatic effect on the responses to hyperpolarisation in the most of the subjects, causing an overshoot in threshold reduction below the original resting level which then returned back to the resting level. A similar phenomenon was described by Raymond (166) when he reported a biphasic response to temperature in frog nerve and can be explained in terms of the sodium pump. The process of warming causes an immediate increase in activation of the sodium pump above its normal level of functioning, which causes excess movement of sodium ions. The resulting fall in intracellular sodium concentration leads to the rate of the sodium pump falling back to equilibrium values.

The effects on membrane potential produced by the sodium pump, exert their influence at the early portion of the response to depolarisation, as well as in later responses in hyperpolarisation and may therefore provide the explanation for the variable changes seen between 5 - 10 ms delay. These changes could also be explained by the variable effect of temperature on the fast potassium conductance that is known to function at this delay (1).
All except Experiment 3 failed to achieve a maintained plateau in skin temperature (Fig 7.3). In this experiment it became evident that in the steady state there is a constant effect in the responses to hyperpolarising and depolarising currents and that this effect is different depending on the temperature. Figure 7.4 demonstrates this effect. The left hand graph shows three superimposed traces taken at the cold plateau and the right hand one shows three traces taken at the hot plateau. The three traces at each plateau are almost identical to the others at the same temperature, whereas the shape of the 'cold' traces is distinct from the shape of the 'hot' traces. Therefore hyperpolarisation is also affected by actual temperature and this effect cannot just be ascribed to the process of warming or cooling.

Thus, the lowering of nerve temperature causes an 'upward' shift in threshold reduction in both the responses to depolarisation and hyperpolarisation. Whereas, warming causes a 'downward' shift in both directions, but with a variable overshoot seen with hyperpolarisation (Fig 7.1C,D and 7.2).

In summary, responses to subthreshold depolarising currents are affected primarily by actual temperature. Moreover, this effect is maximal and correlates between subjects at delays greater than 30 ms and thus indicates involvement of slow potassium channels, such that there is a slowing of accommodation as the temperature is reduced. Changes are also witnessed early on in the trace which may indicate involvement of the sodium pump or fast potassium channels but these changes do not correlate as well between subjects and thus their significance is less certain. Whereas the responses to subthreshold hyperpolarising currents are affected by the actual temperature and the rate of change of temperature. The action of the sodium pump is altered by the change in temperature, but is less affected by the actual temperature once a plateau has been achieved.
We have therefore provided evidence that temperature has a profound effect on the action of the sodium pump and on the action of potassium channels in nerve axons and that the technique of threshold electrotonus provides a unique means of studying these effects.
Figure 7.3: Experiment 3 of the Temperature Protocol

A: Latency changes (in ms) with respect to the skin temperature changes in B. B: Skin temperature (in °C) showing that the ‘cold plateau’ (26°C) was maintained for 20 minutes and the ‘hot plateau’ (35°C) was maintained for 40 minutes.
Figure 7.4: Threshold Electrotonus Waveforms Recorded During Experiment 3 of the Temperature Protocol in a Normal Subject

Threshold changes (top) due to polarising currents (bottom), both expressed as percentages of the control threshold tested with 1 ms pulses. A: Three superimposed recordings while the skin temperature was maintained at 26°C (cold). This shows the typical increase in threshold reduction, maximal between 40 - 70 ms delay. B: Three superimposed recordings while the skin temperature was maintained at 35°C (hot).
8. THRESHOLD ELECTROTONUS AND MULTIPLE SCLEROSIS

8.1 INTRODUCTION

Standard electromyographic examinations in multiple sclerosis (MS) usually show no evidence of abnormality unless coincidental entrapment neuropathy or peripheral neuropathy due to another cause co-exists (167; 168). Extensive demyelination, such as that which occurs in the central nervous system, is apparently not found in peripheral nerve in MS. However, there have been reports of internodes with reduced myelin thickness and reduced internode distance (169). For a significant slowing of conduction velocity, a reduction in myelin thickness of 75% and reduction in internode distance by 50% may be required (170). A number of researchers have studied patients with MS with less standard electrophysiological techniques and have discovered certain abnormalities, which indicate that the peripheral nerve may be involved (171).

Shefner et al (172) found a reduction in the minimum conduction velocity, or in other words, the velocity of the slowest conducting fibres, in the sural nerves of patients with MS. In addition, they showed a significant reduction in the amplitude of supernormality, a finding that had previously been reported by Eisen et al (173). Evidence for an abnormality in the terminal nerve network or in the neuromuscular junction has been suggested with the finding of abnormal jitter when using single fibre electromyography (174; 175). Moreover, it was found that those MS patients that exhibited abnormal jitter also had muscle unit potentials (MUP) that were of significantly longer duration and that
the mean MUP area and amplitude were significantly increased. Prolongation of the relative refractory period when using paired stimuli described by Hopf and Eysholdt (176), provides further evidence for abnormal peripheral nerve in MS.

The present study was prompted by an investigation of motor axon excitability in amyotrophic lateral sclerosis (ALS), employing the method of threshold electrotonus (21). The ALS patients in this preliminary clinical study exhibited either heightened excitability starting 5-10ms after the onset of the current (Type 1 response), or an abrupt reduction in excitability (Type 2 response). Applying the technique to rat nerve and a computer model of human motor axon, both changes were attributable to a deficiency in fast and slow potassium channels (1)

Two of the four MS patients used as neurological controls in the above study produced an abnormal waveform distinct from those seen in the ALS patients or in any of the other neurological controls. We therefore examined a larger series of patients with MS to assess the reproducibility of this finding and have correlated the neurophysiology with biochemical inflammatory markers found in the disease.

8.2 METHODS

8.2.1 Subjects

Forty-eight patients were selected from the wards and the out-patient department at The National Hospital for Neurology and Neurosurgery, on the basis of a diagnosis of clinically definite MS with laboratory support (age range 26-63 years; mean age 40.1 years). A full clinical history and examination was undertaken on each patient. A conventional nerve conduction study and electromyographic examination was carried out on those patients who had symptoms that might have been attributable to a peripheral neuropathy. This included
sensory and motor amplitudes, velocities and latencies in symptomatic nerve territories and appropriate needle examination. The thirty-five normal control subjects were members of staff at The National Hospital and The Institute of Neurology with no record of neurological pathology (age range 22-60 years; mean age 36.5 years).

In addition, ten of the patients with MS were tested on at least two separate occasions, a month apart. These were ten consecutive patients seen at the hospital and were not chosen on the basis of their clinical history or threshold electrotonus results. Urine and serum collections were made on each occasion to assess the levels of various inflammatory parameters, including urine neopterin-creatinine ratio (UNCR) (177), C-reactive protein (CRP), vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1).

The threshold electrotonus recordings and the taking of urine and serum samples were made with informed consent, and the study was approved by the Joint Hospital and Institute Ethics Committee.

8.2.2 Threshold Electrotonus Recordings

The method of recording threshold electrotonus was essentially the same as has been described in Chapter 2. Compound muscle action potentials (CMAPs) were recorded by surface electrodes from abductor digiti minimi (ADM), by stimulation of the ulnar nerve at the wrist, as previously reported. In addition, most patients had recordings made contralaterally and also proximally, stimulating the ulnar nerve above the elbow.

In the case of the MS patients, ulnar nerve excitability was tested by alternating just two levels of polarising current of 100ms duration as the abnormalities previously seen were only present in the responses to the 40% depolarising current. Three stimulus conditions were therefore tested in turn: 1 ms test stimulus alone (control), and the test stimulus
superimposed on the 100 ms polarising currents set to 40% and -40% of the last control stimulus.

As in previous experiments, excitability changes due to the polarising currents were expressed as percentage reduction of the control threshold. The interval between the polarising and test pulses was used as the abscissa for the threshold electrotonus plots. All the waveforms displayed during recording were saved on optical disk (Panasonic WORM 1.4GB), including the raw EMG data, and could be regenerated off-line, to check on the tracking and allow measurement of other parameters, such as the initial threshold.

8.2.3 Assays

All assays were carried out by Dr Gavin Giovannoni in the Department of Neuroimmunology at The Institute of Neurology.

Urine neopterin and creatinine were measured by high pressure liquid chromatography (HPLC) (178), using a reverse-phase Anasil 80, 5μm ODS column (Anachem). Elution was performed using degassed 15mmol/L potassium phosphate buffer, pH 6.4, at a flow rate of 1 ml per minute. Neopterin detection was by native fluorescence using an excitation wave length of 353nm and an emission wavelength of 438nm. Creatinine was measured using UV absorption at 235nm. The fluorescence and UV detector were linked serially to allow the determination of neopterin and creatinine on the same chromatographic run. Urine samples were prepared by diluting them 1 in 10 with the 15mmol/l potassium phosphate buffer, pH 6.4, containing 5.4mmol/l ethylenediaminetetraacetic acid (EDTA) to dissolve any urinary sediments. A fixed volume sample loop of 20μl was used.

ICAM-1 and VCAM-1 were measured by standard sandwich enzyme-linked immunosorbent assays (ELISA), using commercially available mouse monoclonal antibody pairs marketed for ELISAs (Serotec, Oxford). The assays were developed according to the manufacturers
guidelines with some minor modifications. The second or detector antibodies were biotinylated and conjugated to a streptavidin-biotin horseradish peroxidase complex (Amersham). O-phenylene diamine (Sigma) was used as the enzyme substrate. The assay was calibrated using a secondary standard of pooled sera from patients with active rheumatoid arthritis with elevated ICAM-1 and VCAM-1 levels. This standard had been calibrated using commercial ICAM-1 and VCAM-1 ELISAs (Quantikine™, R & D Systems Europe). CRP was measured using a standard radial diffusion immunoassay.

8.3 STATISTICAL ANALYSIS

The threshold electrotonus data was plotted and analysed with programmes already written for the purpose by Bostock. Further data was analysed using Student t-tests and f-tests to assess inter- and intra-patient variability and correlation coefficients were calculated to assess the relationship between threshold reduction and the pro-inflammatory factors. Statistical significance was taken as p < 0.05.

8.4 RESULTS

In the patients that had standard nerve conduction and EMG examinations carried out, all the results were within the normal range.

Figure 2 compares threshold electrotonus recordings from the normal controls and the MS patients, stimulating the ulnar nerve at the wrist. Thirty-five control recordings are superimposed (Fig. 8.1A). The waveforms correspond to previous single motor unit and CMAP recordings of threshold electrotonus from normal subjects (1;21) revealing a tight range of responses to the depolarising current and a more widespread range of responses to hyperpolarisation. Figure 8.1B shows the superimposed traces of sixty recordings from forty eight MS
patients and exhibits a similar pattern to the normal controls. The mean waveforms of the MS and normal control groups, shown in figure 8.1C, revealed a slight divergence at 50 - 70 ms delay, but fell short of statistical significance (p = 0.06, t-test). However, the variability of the depolarising responses in the MS patients was significantly greater than in the normal controls at this delay (p < 0.02, f-test).

To investigate the reason for the variability of the responses at 50-70ms after the start of the 40% depolarising current, we plotted the threshold reductions at this delay (Fig. 8.2). A sub-group of 16 MS patients exhibited greater excitability outside the range of values from the normal controls and the rest of the MS group. The full waveforms of the individual subjects were subsequently studied and it was found that the waveforms of these 16 patients (“high MS”) differed in shape from the normal control group and those MS patients in the normal range (“normal MS”) (Fig 8.3A). The difference between the “high MS" and “normal MS" recordings was restricted to the responses to depolarisation and was qualitatively distinct from the difference between the control recordings with high or normal excitability at 50-70ms (Fig 8.3B). The “high MS" group in this study closely resembled the two abnormal MS recordings in the previous ALS study (21).

Studies comparing responses from opposite sides showed no evidence of asymmetry (Fig 8.4), except in one patient who had clinically mild MS with symmetrical symptoms of paraesthesiae only and who had a normal standard EMG. Of the 8 patients whose left and right responses were recorded, 5 were in the “high MS" group and of these 4 produced abnormal waveforms from both wrists. The fifth was the patient described above whose response from the right wrist was abnormal but that from the left was within the normal range. The remaining patients produced symmetrical results within the normal range. Of particular
Figure 8.1: Threshold Electrotonus Waveforms Comparing Normal Controls and Patients with Multiple Sclerosis

Threshold changes (top) due to polarising currents (bottom), both expressed as percentages of the control threshold tested with 1ms pulses. 

A: Superimposed responses to +/- 40% polarising currents from 25 normal control subjects. 
B: Superimposed responses to +/- 40% polarising currents from 60 recordings in 48 MS patients. 
C: Superimposed mean responses of the normal control subjects (thin lines) and the MS patients (thick lines)
Figure 8.2: Threshold Reduction 50–70 ms after 40% Depolarising Currents Comparing Normal Controls and Patients with Multiple Sclerosis

Percentage threshold reduction at 50–70 ms delay after 40% depolarising currents. There is a significantly greater variability in the MS patients at this delay ($p < 0.02$). The thick horizontal lines represent the mean threshold reductions, while the dashed line separates the 16 MS patients outside the normal range.
Figure 8.3: Mean Threshold Electrotonus Waveforms Comparing Subgroups of Normal Controls and Patients with Multiple Sclerosis

Threshold changes (top) due to polarising currents (bottom), both expressed as percentages of the control threshold tested with 1ms pulses. A: Superimposed mean responses to +/- 40% polarising currents comparing normal controls (mean NC) with those 16 MS patients with increased excitability at 50 – 70 ms (high MS) and the remaining MS patients (normal MS). The normal MS and mean NC groups superimpose exactly, whereas the high MS group produces a clearly distinct waveform. B: Comparison of mean responses of the normal controls (mean NC) with those normal controls with threshold reductions at the upper end of the normal range (high NC).
Figure 8.4: Threshold Reduction 50 –70 ms after 40% Depolarising Currents Comparing Left and Right Hands in Patients with Multiple Sclerosis

Percentage threshold reduction at 50 – 70 ms delay after 40% depolarising currents. The bilateral responses from each individual patient are linked. The unlinked points represent the one patient who showed asymmetrical responses. The arrow indicates the patient with hemiatrophy (see text). The thick horizontal line represents the level above which the 'high MS' group were found.
Figure 8.5: Mean Threshold Electrotonus Waveforms Comparing Responses at ADM Following Stimulation of the Ulnar Nerve Above the Elbow.

Threshold changes (top) due to polarising currents (bottom), both expressed as percentages of the control threshold tested with 1ms pulses. The mean responses from above elbow stimulation from the normal controls, patients in the 'high MS' group and the whole MS group are shown. The depolarising responses superimpose and show no difference, comparable to that found at the wrist (cf. fig 8.1C).
note, was that in one patient with MRI-proven hemi-atrophy of the spinal cord and corresponding hemiparesis, responses were clearly symmetrical from both wrists.

No comparable difference in waveform was found with more proximal stimulation of the ulnar nerve at the elbow (Fig 8.5). The abnormality was only found with distal stimulation.

The abnormality in threshold reduction in the MS patients was found to vary not only between patients, but also with time within a single patient. In ten patients tested, on at least two occasions, a month apart, the differences between the first two recordings were much more variable than those from normal control subjects (p < 0.04 f-test, comparing the difference in threshold reduction at 50 - 70 ms). Figure 8.6 shows that this abnormal variability was mainly due to the very large changes in accommodation in three of the patients. In these three patients studied on more than one occasion, the changes in threshold electrotonus waveform appeared to parallel the changes in clinically assessed disease activity. Of particular note was one patient who, as a result of two episodes of relapse and remission which occurred within a few months, was seen on multiple occasions.

8.4.1 Case History (Subject MR):

A 32 year old gentleman with a seventeen year history of relapsing-remitting multiple sclerosis presented with a four day history of worsening gait such that he could only take a few steps with a stick. He had had a mild spastic paraparesis prior to presenting but had been fully mobile. It was felt that he had a spinal cord relapse and he was treated with intravenous methylprednisolone (1g daily for 3 days). Within two months he had returned to his previous level of functioning. A month later he presented with reduced vision in the
Figure 8.6: Difference Between Threshold Reduction at 50 – 70 ms at Two Sequential Recordings From Normal Controls and Patients with Multiple Sclerosis

There is a significantly greater variability in the MS patients ($p < 0.04$) than the normal controls.
Figure 8.7: Four Consecutive Threshold Electrotonus Waveforms from the Same Patient with Multiple Sclerosis (see case history)

Threshold changes (top) due to polarising currents (bottom), both expressed as percentages of the control threshold tested with 1ms pulses. A: During his relapse with a myelopathy, the waveform is abnormal (thick line) with poor accommodation most marked between 50 – 70 ms (loss of concavity of the trace). Whereas, this returns to normal (thin line) with improvement of his symptoms following steroids. B: During his relapse with optic neuritis, the waveform once more becomes abnormal (thick line), with a return to normal (thin line) with the return of his visual acuity.
left eye, which was diagnosed as optic neuritis. This resolved spontaneously within a month.

His electrotonus waveforms are shown in Figure 8.7 and reveal the typical MS abnormality during the episodes of relapse with return to a normal pattern during the remissions.

To test whether the variability seen in the responses of patients with MS was related to the inflammatory activity in the disease, we compared the threshold electrotonus recordings with the following inflammatory markers: CPR, ICAM-1, VCAM-1 (177;179) and UNCR (177). In the ten consecutive patients, who had at least two recordings at monthly intervals, a positive correlation was found between the threshold reduction at 50-70ms delay and the urine neopterin-creatinine ratio (r = 0.53; p < 0.02) (Fig. 8.8). It is noteworthy that the regression line passes close to the point where the mean normal values would be expected to lie: The mean normal control threshold reduction was 47.04% in this study; and the mean normal control UNCR was 133.6 μmol/mol from (177). However, urine and serum samples were not taken from our control subjects. There was no significant correlation with CRP, VCAM-1 or ICAM-1.

8.5 DISCUSSION

The technique of threshold electrotonus was developed to provide information about potassium channels and accommodative processes in normal human axons (1;180). The responses to depolarising currents show very little variability in normal controls and also in most neurological controls studied so far, which includes patients with various neuropathies, neuronopathies and central nervous system diseases. However, abnormal accommodation to subthreshold depolarising currents has been found in patients with motor neurone disease, which
Figure 8.8: Relationship Between the Threshold Reduction at 50–70 ms and the Urinary Neopterin–Creatinine Ratio (UNCR) in Patients with Multiple Sclerosis.

There is a positive correlation between the threshold reduction at 50–70 ms and the UNCR in patients with MS ($r = 0.53; P < 0.02$).
was evident 10 - 20 ms after the start of the current (21). This observation has been confirmed in a second study, which, in addition, found an abnormal response to hyperpolarising currents in diabetic patients, although the abnormalities were only evident in averaged recordings (181).

A third abnormality of threshold electrotonus, (first seen in two out of four MS patients included as neurological controls in the first MND study) has been confirmed in the present study. These results demonstrate that although the majority of MS patients have nerves which accommodate normally to depolarising currents, a significant minority show a distinctive pattern of altered accommodation. The abnormality, is seen most clearly in Fig 8.3A, and consists of increased excitability (or reduced accommodation) to depolarising currents, occurring much later that that in ALS and most pronounced at 50-70 ms after the start of the current. Since this abnormality only affected the later part of the depolarising response and left the hyperpolarising response unaffected, we conclude that a slowly activated, voltage-dependent conductance must have been affected. The obvious candidate is the slow potassium conductance, sensitive to tetraethyl ammonium (TEA), previously shown to be responsible for the slow accommodation to depolarising currents in rat axons (1;11).

In those patients who had repeated studies, it was shown that the presence of this abnormality fluctuated in three of the patients producing a change from an abnormal to normal waveform. From the clinical histories and examinations there appeared to be a relationship between relapse or clinically active disease and the electrotonus.

It therefore appears that there is specific abnormality of the slowly activating potassium channels in a proportion of patients with MS and that this fluctuates with time. This abnormality appears to be confined to distal recordings (Fig. 8.5).
The reasons for the ion channel disturbance in the MS patients remains unclear. However this study indicates that there is a positive correlation between the channel dysfunction and the inflammatory activity in the disease as measured by the urinary neopterin-creatinine ratio. Increased neopterin production, which occurs in MS (177;182-185) is a sensitive marker of interferon gamma - (IFNγ) and tumour necrosis factor alpha - (TNFα) induced macrophage activity (186). Therefore the correlation between the levels of urinary neopterin excretion and the threshold reduction suggests a relationship between pro-inflammatory mediators and a reversible dysfunction of slow potassium channels. Recent clinical observations (187) and experimental evidence (188) suggests that a number of cytokines including TNFα and IFNγ, can directly impair nerve conduction in vivo. Although the mechanisms are unknown, the administration of TNFα reduces potassium and sodium conductance in neurones of *Aplysia kurodai* (189;190). Therefore, it appears that cytokines may act on excitable membranes to produce the type of phenomena that we have described. The lack of correlation between the threshold reduction and the soluble adhesion molecules, VCAM-1 and ICAM-1, and the acute phase protein CRP is not unexpected, as their levels are intermittently elevated in patients with MS and the time course of their production in relation to clinical disease activity is poorly defined (179;191)

As has been described in Chapter 7, the region of the trace, which corresponds to slow potassium conductance, is also affected by temperature. As the temperature is lowered to levels well below normal a very similar abnormality to that which was found in the MS patients is seen. Unfortunately, at the time of these experiments the effects of temperature had not been elucidated and thus routine temperature recordings were not made on the MS subjects.

Although an abnormality similar to that found in MS has been seen in normal control subjects and patients with other neurological conditions,
in the majority of cases, the marked variability with time found in the MS subjects does not occur. One possible explanation is that patients with MS have a more variable skin temperature than the other subjects and may represent an autonomic or hypothalamic phenomenon related to relapse. Alternatively, it is possible that MS patients react abnormally to changes in temperature. However a temperature protocol was carried out on subject MR, who had previously shown marked variability in his threshold electrotonus recordings with time, and he was found to respond identically to the normal control subjects (see Chapter 7). At the time the temperature experiment was carried out, subject MR was in remission and his resting threshold electrotonus trace was normal. It is therefore possible that he might have responded differently if he had been tested during a relapse.

Further evidence that the abnormality found in the MS patients is not restricted to an effect of temperature is provided if one considers the case of one of the normal control subjects (GS). He had no evidence of any neurological disease, but was found to have abnormal resting threshold electrotonus traces. Figure 8.9 represents a typical threshold electrotonus trace taken from subject GS at a time that his skin temperature was 33°C and therefore relatively warm. This is superimposed on the mean trace from all the normal control subjects. What is most evident from this figure is the similarity between the responses to all but the 40% depolarising current, which exhibits the classical abnormality seen in MS patients or subjects with cooled limbs.

Moreover, subject GS, when exposed to a full temperature protocol, showed all the expected responses to changes in temperature, with an increase in threshold reduction to depolarisation and a decrease in threshold reduction to hyperpolarisation on cooling and the reverse on warming. Thus the abnormality seen in MS patients is not synonymous with a cold nerve.
Figure 8.9: Mean Threshold Electrotonus Waveforms Comparing the Mean Normal Control with Normal Control Subject GS

Threshold changes (top) due to polarising currents (bottom), both expressed as percentages of the control threshold tested with 1ms pulses. The recording from GS was taken when his skin temperature was 33°C. The responses to all but the 40% depolarising currents are similar to the mean normal control trace.
Lastly, there was a positive correlation between clinically assessed relapse and the UNCR, which monitors inflammatory activity in the disease. Thus MS patients only demonstrated the abnormal pattern in their threshold electrotonus trace when in relapse.

A similar abnormality in threshold electrotonus, also attributed to slow potassium channel dysfunction, has recently been reported in monomelic amyotrophy with spinal hemiatrophy (MASH, Hirayama's diseases) (192). This suggested that the abnormality might be related to spinal cord or anterior horn cell involvement in the disease. However, one of our MS patients had MRI-proven hemiatrophy of the spinal cord, including the cervical and thoracic segments responsible for ulnar nerve innervation, and yet his threshold electrotonus traces were within the normal range and the responses from the affected and unaffected limbs were indistinguishable.

In conclusion, we have found evidence for a distinctive peripheral nerve abnormality in MS, detectable in about a third of patients at any one time, which fluctuates within a given patient, apparently in parallel with the inflammatory disease process. It is not clear whether the abnormality provides a reflection of pathological processes affecting the central nerve fibres, or whether it is an unrelated secondary phenomenon, but it is probably unlikely to be a temperature effect. However, if there is any relationship to the pathophysiology of MS, threshold electrotonus may provide a useful, non-invasive means of monitoring disease progress and response to treatment.
9. DISCUSSION AND CONCLUSIONS

Further to the initial findings of Bostock et al (21), the current study was set up to further assess the usefulness of threshold electrotonus in a clinical setting.

The first aim of the study was to assess whether threshold electrotonus might provide a specific and sensitive diagnostic test for MND, as current neurophysiological tests fall short in this respect.

The initial study found that all patients with MND who were tested exhibited one of two abnormal responses, Type 1 and 2, and it was felt that those patients exhibiting Type 2 responses had a more rapidly progressing form of the disease. In addition, it was hypothesised that there may be a progression from a Type 1 to Type 2 response as the disease progressed. The second aim of the current study was, therefore, to assess whether threshold electrotonus might be used as a prognostic tool to indicate the rate of progression of the disease.

Finally, with the increasing routine use of the anti-glutamate drug, riluzole in patients with MND, the third aim was to assess whether the effect of this drug on survival was actually due to its action on sodium channels, which might be acting to redress the proposed ionic imbalance. In addition, it was hoped to assess the effect of membrane stabilising medication on the proposed ionic imbalance.

Therefore, 70 patients with MND were tested with threshold electrotonus as a means of assessing the reproducibility of the previous study and assessing the use of the technique as a diagnostic tool in the disease. These recordings were compared to 35 normal controls and 64 patients with other neurological conditions. Neurological control subjects produced threshold electrotonus recordings within the normal range and over time repeated testing produced almost identical responses. There
were two exceptions. First, some of the patients with multiple sclerosis who had traces within the normal range at 10 – 20 ms delay, however demonstrated an abnormality at a later delay, that correlated with clinical relapse and certain inflammatory markers. Secondly, the patient with neuromyotonia, who demonstrated a novel oscillating threshold and produced a threshold electrotonus trace similar to those found in ischaemic nerves.

In patients with multiple sclerosis, the abnormality only affected the depolarising response, 50 – 70 ms after the onset of the current. The obvious candidate for an abnormality at this position, is the slow potassium conductance that is sensitive to TEA. Why there might be a peripheral ion channel abnormality present in patients with a central nervous system disease, cannot be answered by this study. However, the study did indicate a positive correlation between this abnormality and active disease. Threshold electrotonus may therefore be able to provide a non-invasive means of studying disease activity in patients with multiple sclerosis. The cause of the underlying mechanism will require further study.

The testing of the patient with neuromyotonia, who was known to have antibodies directed against potassium channels, provided a unique opportunity to assess abnormalities of potassium conductance. Traces similar to those that had been found in MND were expected. The results proved surprising with the presence of an oscillating threshold and a final trace, which appeared ischaemic. Further studies are currently being carried out on patients with neuromyotonia that seem to rule out ischaemia as a cause of the final trace and a possible explanation is being sought in the context of the potassium channel antibodies. One possible explanation is that the potassium channel antibodies are directed against the potassium channels in the Schwann cells rather than the nerve membrane. This would lead to an increase in potassium concentration under the myelin, in the periaxonal region, and
could explain the clinical and electrophysiological findings in neuromyotonia.

Of the 70 patients with MND that were studied, only 28% of them exhibited an abnormality (18 patients - "type 1" and 2 patient - "type 2") when stimulating the ulnar nerve at the wrist. Recordings within the normal range were found in the majority, unlike the patients tested in the original study (21). Moreover, the abnormalities were only found in MND patients with lower motor neurone signs in the limbs i.e. the 'ALS' and 'PMA' subgroups. Recordings taken from both wrists were identical in all cases, except the one patient outlined in chapter 5. Recordings from nerves other than the ulnar nerve were limited in number but all these were within the normal range.

Although the mean age of the normal and the MND groups differed, the previous study revealed no effect of age on the threshold electrotonus waveform and the current study confirmed these findings when comparing the older subjects with the younger ones.

Serial recordings in 37 patients with MND, showed that the majority of patients exhibited little change with time. There were four patients who showed marked increase in their threshold reduction between 10 – 20 ms delay and these were all patients with rapidly progressive ALS, who died during the course of the study. However, all the patients with initial recordings in the normal range, remained within this range, including the four patients mentioned above, despite marked progression of their disease at various rates, assessed clinically and using a modified Appel rating scale (see appendix 1).

Patients who initially presented with a Type 1 abnormality showed minimal change with time, except for one patient whose threshold electrotonus normalised. A further exception was illustrated by the one patient with a strikingly asymmetrical and slowly progressive disease process, who initially exhibited a Type 2 response in the affected hand.
This then converted to a Type 1 response at subsequent recordings. These changes may be explained in terms of the death of unstable motor units, with normal or different unstable units being recorded at subsequent testing.

A new phenomenon was discovered during the longitudinal recordings of patients with MND. Three patients developed flattening of the early part of the response to depolarisation. An explanation for this pattern of abnormality is uncertain, but may indicate partial loss of fast and slow potassium channels and would require further in vitro and computer model experiments to clarify.

Considering the drug treatment arm of the study, riluzole, which has actions on sodium channels, in addition to its anti-glutamate activity, did not appear to have a membrane stabilising effect on the nerve as assessed by threshold electrotonus. The majority of the patients showed variability with time of their threshold electrotonus waveforms that were similar to the normal controls, despite treatment with riluzole. One patient on riluzole exhibited a marked increase in threshold reduction between 10 – 20 ms and died of his rapidly progressive ALS during the course of the study.

Unfortunately, after necessary exclusions, the arm of the study aimed at assessing the effect of membrane stabilising medication on threshold electrotonus, amounted to a single case report. The inclusion criteria required patients not to be taking riluzole and to have an abnormal initial threshold electrotonus recording. Only two patients fitted both criteria and one of patients died prior to taking the drug. The one patient who did take carbamazepine, had a sustained normalisation of his threshold electrotonus recordings. He also noticed a marked reduction in his fasciculations and has continued to take the drug. His disease has continued to progress, but slowly. Despite the onset of mild bulbar features, two years after the end of the study, he has almost no fasciculations either on clinical or electrical examination. Whether the
carbamazepine has also had any effect on the speed of his progression, can not be surmised from this study. However, it does provide a possible avenue for further trials on a larger number of patients in the future.

Thus, threshold electrotonus appears to be a specific test for subgroups of MND affecting the lower motor neurone, however it is not sufficiently sensitive to be used routinely as a diagnostic test. The lack of sensitivity produces the first barrier to the use of threshold electrotonus as a prognostic test. The proposed ion channel imbalance (21) is not uniformly present in patients with MND, even in those with rapidly progressive disease. In the cases where the abnormalities were found, there was no correlation with the disease progress. From the large multicentre trials of riluzole (65;66;193) there does appear to be a survival benefit, but I could not demonstrate any evidence that this was working via mechanisms in the ionic channels of the nerve membranes.

The possible mechanism of the abnormality found in some patients with MND remains elusive. Computer models of human nerve and in vitro studies (21) confirm that Type 1 and 2 abnormalities relate to abnormalities of potassium channel function. However, the current study found that only a minority of patients show these abnormalities. Only patients with lower motor neurone signs as part of their disease process (ALS and PMA), demonstrated such changes. That subgroups with lower motor neurone signs and fasciculations might be more likely to show evidence of membrane instability is unsurprising. However, the failure to record such changes in the majority of patients within these subgroups, in whom fasciculations were very apparent, is surprising. There were no particular features in the history, examination or standard neurophysiology of those patients with abnormal threshold electrotonus recordings, which differentiated them from the majority, who fell within the normal range. As only a few patients exhibited abnormal results, it is likely that the abnormalities recorded are a secondary phenomenon.
There is also no corroborating evidence to confirm the hypothesis that the presence of the abnormalities contributes to the mechanism of cell death in motor neurone disease.

The development of the use of the technique of threshold electrotonus in the clinical context of MND, arose from the hypothesis that fasciculations indicated the presence of membrane instability. The technique provides a unique means of studying this hypothesis. It is known that fasciculations arise at many different sites within the motor unit (43-47), and it is possible that the instability causing the fasciculations in patients with normal threshold electrotonus recordings was distant to the site of the recording. Alternatively, there may be a particular feature of the unstable terminal sprouts that is recorded during the threshold electrotonus protocol that produces an abnormal trace. Whatever the underlying mechanism, there is no clear explanation as to why the original study produced such consistently abnormal results in the MND patients studied. There were no apparent differences in the history, examination, presence of fasciculations, or standard neurophysiological examination that might explain the marked variance of the results in the two studies.

Although threshold electrotonus may have its limitations in the clinical context of MND, it is a powerful tool for investigating excitable membranes in vivo. It can provide important information about membrane properties in normal and diseased nerve. The current study has generated further questions, which require investigation. The mechanisms of the intermittent abnormalities found in MND and those found in MS, which correlate with disease activity, require further clarification. Neuromyotonia is an ideal disease to study using this technique, as the primary abnormality involves potassium channels. However, at present, theories derived from in vitro and computer models cannot explain the unusual findings in this disease. Further studies are in progress.
Currently, the greatest value of threshold electrotonus is in the investigation of pathophysiological mechanisms that cannot be investigated by any other technique and a clinical role may emerge through further study, especially for metabolic and toxic neuropathies in which there are uniform abnormalities within the nerve.
10. REFERENCE LIST


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11. APPENDIX 1

11.1 MODIFIED APPEL SCORE

The following clinical scoring system was modified from the rating scale created by Appel V et al (137). The modifications were made to facilitate recordings made in patients' homes, in order to minimise on equipment that needed to be transported.

A minimum score of 22 points indicated normal function and a maximum score of 125 points indicated severe disability.

**Bulbar Function (6 – 30 points)**

Swallowing:
- General diet 3
- Soft diet (soft cooked) 6
- Mechanical soft diet (finely chopped or ground + thick liquids) 9
- Pudding consistency diet (strained, pureed, blended + thick liquids) 12
- Tube feeding 15

Speech:
- Clear 3
- Slightly slurred for pa/ta/ka 6
- Slurred 9
- Unintelligible 12
- None 15
**Respiration: (6 – 24 points)**

- Normal 6
- Dyspnoea on moderate exertion (more than routine activities) 12
- Dyspnoea on mild exertion (routine activities e.g. wash, dress, walk 1 mile, 2 flights stairs) 18
- Ventilatory/ respiratory device, some/all of the time 24

**Muscle Strength: (4 - 28 points)**

(MRC grading – deltoïd, biceps, triceps, wrist extension, finger flexion
- iliopsoas, quadriceps, hamstrings, ankle dorsiflexion, ankle plantar flexion, toe extension and flexion)

**Muscles of upper limb (sum of right and left sides)**

<table>
<thead>
<tr>
<th>Score Range</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>2</td>
</tr>
<tr>
<td>62-69</td>
<td>4</td>
</tr>
<tr>
<td>54-61</td>
<td>6</td>
</tr>
<tr>
<td>46-53</td>
<td>8</td>
</tr>
<tr>
<td>32-45</td>
<td>10</td>
</tr>
<tr>
<td>18-31</td>
<td>12</td>
</tr>
<tr>
<td>&lt;17</td>
<td>14</td>
</tr>
</tbody>
</table>

**Muscles of lower limb (sum of right and left sides)**

<table>
<thead>
<tr>
<th>Score Range</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>2</td>
</tr>
<tr>
<td>62-69</td>
<td>4</td>
</tr>
<tr>
<td>54-61</td>
<td>6</td>
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<tr>
<td>46-53</td>
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<td>32-45</td>
<td>10</td>
</tr>
<tr>
<td>18-31</td>
<td>12</td>
</tr>
<tr>
<td>&lt;17</td>
<td>14</td>
</tr>
</tbody>
</table>
**Muscle Function – Lower Limbs: (4 – 23 points)**

**Standing from chair (seconds)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>1</td>
</tr>
<tr>
<td>1.5-3</td>
<td>2</td>
</tr>
<tr>
<td>3.5-5</td>
<td>3</td>
</tr>
<tr>
<td>&gt;5</td>
<td>4</td>
</tr>
<tr>
<td>unable</td>
<td>5</td>
</tr>
</tbody>
</table>

**Walking 20 feet (6m) (seconds)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;8</td>
<td>1</td>
</tr>
<tr>
<td>8.5-12</td>
<td>2</td>
</tr>
<tr>
<td>12.5-16</td>
<td>3</td>
</tr>
<tr>
<td>&gt;16</td>
<td>4</td>
</tr>
<tr>
<td>unable</td>
<td>5</td>
</tr>
</tbody>
</table>

**Need for devices**

<table>
<thead>
<tr>
<th>Device Description</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td>AFO/cane/boots</td>
<td>2</td>
</tr>
<tr>
<td>Walker, crutches and/or wheelchair</td>
<td>3</td>
</tr>
<tr>
<td>Confined mostly/always to wheelchair</td>
<td>4</td>
</tr>
<tr>
<td>confined to bed</td>
<td>5</td>
</tr>
</tbody>
</table>
Hips and legs

Walks and climbs stairs without assistance 1
Walks and climbs stairs with aid of rail 2
Cannot climb stairs but walks unassisted and rises from chair 3
Cannot climb stairs but walks unassisted with AFO / cane 4
Cannot climb stairs but walks with minimal assistance or uses crutches or walker 5
Cannot climb stairs but walks with crutches or walker with assistance or walks with total support 6
Confined to wheelchair 7
Confined to bed 8

Muscle Function – Upper Limbs: (2 – 10 points)

Dress and Feed

Independent 1
Independent with aids (button hooks, zip pull) 2
Mini assistance (needs for cutting meat, buttons) 3
Major assistance (most of dressing and/or feeding) 4
Dependent 5
Arms and Shoulders (grading most affected side)

Starting with arms at side, abducts the arms in a full circle until touch above head 1

Raises arms above the head only by flexing the elbow or using accessory muscles 2

Cannot raise hands above head but raises a glass of water to mouth 3

Cannot raise hands to mouth but can use hands to hold articles 4

Cannot raise hands to mouth and has no useful hand function 5