INVITED REVIEW

ABSTRACT: Conventional electrophysiological tests of nerve function focus on the number of conducting fibers and their conduction velocity. These tests are sensitive to the integrity of the myelin sheath, but provide little information about the axonal membrane. Threshold tracking techniques, in contrast, test nerve excitability, which depends on the membrane properties of the axons at the site of stimulation. These methods are sensitive to membrane potential, and to changes in membrane potential caused by activation of ion channels and electrogenic ion pumps, including those under the myelin sheath. This review describes the range of threshold tracking techniques that have been developed for the study of human nerves in vivo: resting threshold is compared with the threshold altered by a change in environment (e.g., ischemia), by a preceding single impulse (e.g., refractoriness, superexcitability) or impulse train, or by a subthreshold current (e.g., threshold electrotonus). Few clinical studies have been reported so far, mainly in diabetic neuropathy and motor neuron disease. Threshold measurements seem well suited for studies of metabolic and toxic neuropathies but insensitive to demyelination. Until suitable equipment becomes more widely available, their full potential is unlikely to be realized. *Muscle Nerve* **21:** 137–158, 1998
Key words: axon; nerve excitability; membrane potential; ion channels; threshold electrotonus

THRESHOLD TRACKING TECHNIQUES IN THE STUDY OF HUMAN PERIPHERAL NERVE

HUGH BOSTOCK, PhD,[1]* KATIA CIKUREL, BSc, MRCP,[2] and DAVID BURKE, MD, DSc[3]

[1] Sobell Department of Neurophysiology, Institute of Neurology, Queen Square, London WC1N 3BG, United Kingdom
[2] Department of Clinical Neurophysiology, The National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, United Kingdom
[3] Prince of Wales Medical Research Institute, Sydney, Australia

Received 6 May 1997; accepted 25 July 1997

Joseph Bergmans[8] 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, and that considerable information could be inferred about the physiology of the axon by measuring the changes in threshold induced by impulse activity or by artificial polarization. The great value of threshold measurements is that changes in threshold usually provide an accurate indication of changes in membrane potential. Bergmans' thesis was a remarkable technical and intellectual achievement, but it was not followed up for many years. Part of the reason for this was probably the difficulty of his method, which involved first functionally isolating a single unit with surface electrodes, and then determining its threshold manually as the voltage which would excite it in three out of five stimuli.

A solution to the difficulty of threshold determination was provided by Raymond's ''threshold hunter,''[66] a feedback circuit which adjusted stimulus duration automatically to keep the probability of exciting an axon (in his case a frog axon in vitro) close to 50%. The term ''threshold tracker'' was subsequently introduced for a similar device, which worked by altering current amplitude rather than stimulus duration.[22] The difficulty of isolating single units can be avoided by tracking the ''threshold'' of a compound action potential, i.e., the stimulus required to produce a compound action potential of a specified size. A compound action potential thresh-

*Correspondence to: Professor H. Bostock
Contract grant sponsor: Motor Neurone Disease Association

CCC 0148-639X/98/020137-22

old generally behaves in exactly the same way as the threshold of a single unit,[30,88] but can be determined for sensory as well as motor fibers, and in patients with neuropathies or neuronopathies as well as normal subjects.

Threshold measurements are essentially complementary to conventional electrophysiological tests of nerve function. They are insensitive to the number of conducting fibers and their conduction velocity; they test the nerve at a point, rather than over a length; and they test the properties of the axonal membrane rather than the integrity of the myelin sheath. Also, unlike measurements of conduction velocity, the resting value of threshold is usually of much less interest than the changes produced by maneuvers which alter the membrane potential, such as ischemia, impulse activity, or applying currents. These changes in threshold, when expressed as percentages, are parameters that can be compared directly between single-fiber and multifiber preparations, between different sites in the same nerve, between subjects, and even between species. Threshold tracking techniques are still little used in clinical neurophysiology, partly because no commercial electromyograph (EMG) yet provides for this type of measurement. The aim of this review is to describe the range of threshold tracking techniques that have been developed for the study of human nerves in vivo, with special emphasis on their clinical applications, to show that these techniques are not difficult but can provide unique information about nerve function. It is also hoped to provide a stimulus to equipment manufacturers to make the facilities for these measurements more widely available.

MATERIALS AND METHODS

A simple threshold-tracking experiment is illustrated schematically in Figure 1 (see also Figs. 2, 3). A 1-ms stimulus is applied to the ulnar nerve at the wrist at regular intervals (e.g., 1 s^{-1}), the compound muscle action potential (CMAP) is recorded from the hypothenar muscle (abductor digiti minimi), and the stimulus stepped up until a maximum response is recorded. A target response is then set to a standard fraction (e.g., 40%) of maximum, and then the stimulus is automatically decreased in steps until the response falls below the target. Thereafter the stimulus is automatically stepped up or down depending on whether the previous response was less or greater than the target response. Threshold tracking with

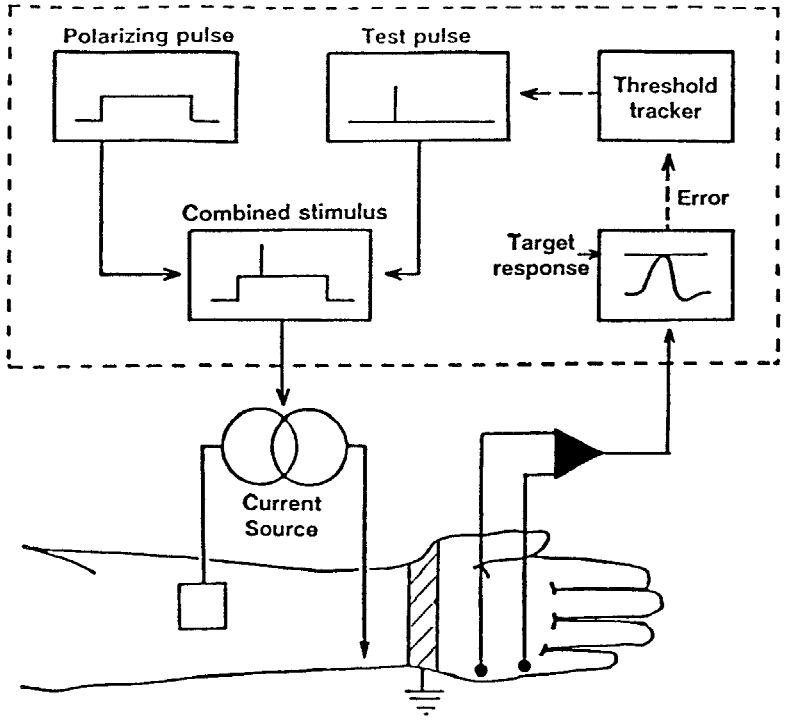

FIGURE 1. Schematic diagram of typical threshold tracking experiment. Functions within broken box are carried out by personal computer. Compound muscle action potential (CMAP) recorded from hypothenar muscle is compared with target response (typically 30–40% of maximal response) and error signal is used to alter amplitude of test stimulus pulse, which is applied to the ulnar nerve at the wrist via an isolated voltage-to-current converter. The test stimulus is optionally combined with a conditioning pulse, e.g., a subthreshold polarizing current, to alter the membrane potential and threshold (see Fig. 2).

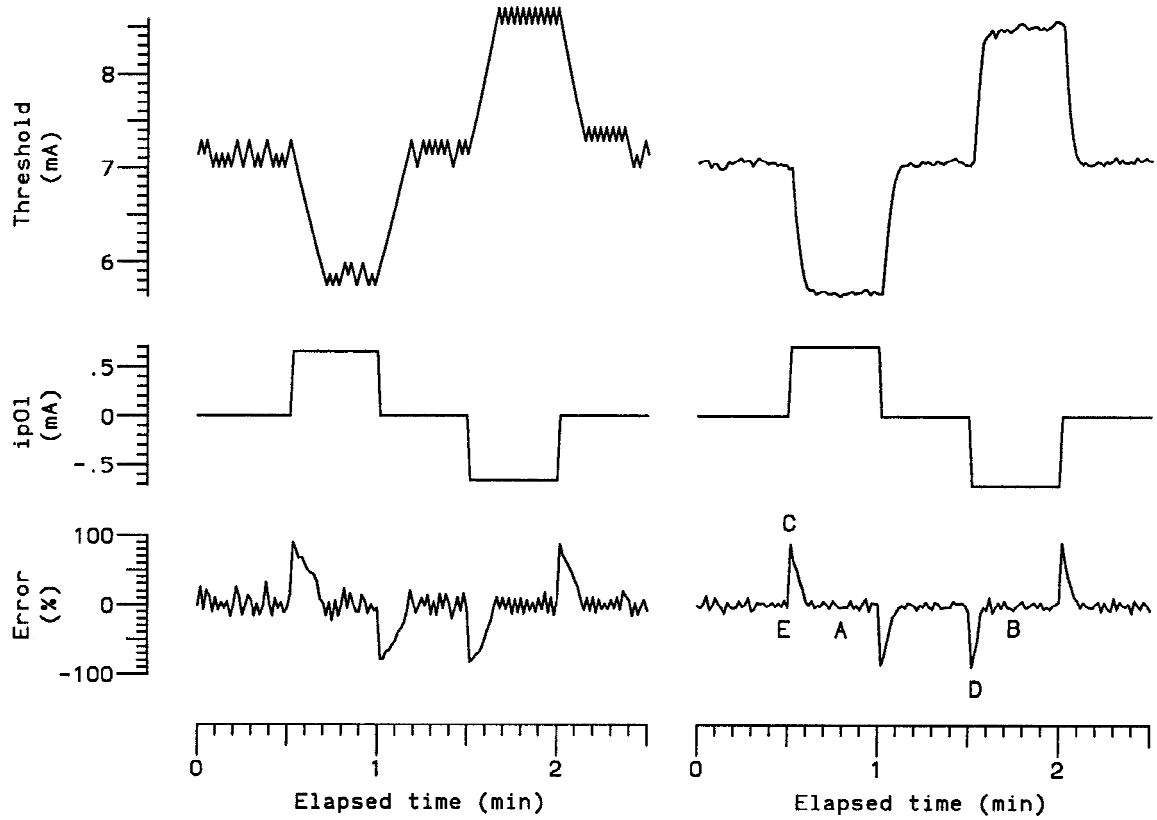

FIGURE 2. Comparison between threshold tracking with fixed and proportional steps. Top: threshold currents recorded as in Figure 1 for 40% CMAP response, tracking (left) with fixed current steps (2% of threshold), and (right) with step size proportional to error (8% for 100% error). Stimulus duration 1 ms. Middle: polarizing current amplitude (ca. ± 10% of resting threshold). Test stimulus applied 20 ms after start of polarizing current. When polarizing current is positive (i.e., in the same direction as the stimulus current) axons are depolarized at the site of stimulation. Bottom: response error, i.e. (response amplitude − target response)/target response × 100. Letters A-E indicate times of traces in Fig. 3. Errors are smaller and tracking is faster with proportional steps.

2% steps is illustrated in Figure 2 (left). Having established the resting (control) threshold, the nerve excitability is then changed in some way: by altering the nerve environment, or by applying additional currents. These may be suprathreshold for the fibers being studied, in which case it is primarily the nerve impulses which cause the change in excitability, or they may be subthreshold, to change membrane potential without triggering impulses. Figure 2 illustrates the effect of subthreshold polarizing currents, starting 20 ms before the test pulse, set to ±10% of the amplitude of the control threshold current. As is usually the case, depolarization increases excitability and reduces threshold, while hyperpolarization increases threshold. Threshold tracking always involves a trade-off between speed and accuracy. In Figure 2 (left) the 2% step size is too large to determine the resting threshold very accurately, but too small to allow a quick response to the change in threshold. These limitations can be minimized by making the step size proportional to the ''error'' (i.e., to the difference between the actual response and target response, as in Fig. 2 right).

The simple experimental protocol in Figure 2, like other threshold comparisons, can be elaborated in two ways. First, to allow for any drift in thresholds, and to minimize electrode polarization, the three stimulus conditions (1, test stimulus alone; 2, test stimulus + depolarizing current; and 3, test stimulus + hyperpolarizing current) can be given in a repeating sequence (1,2,3,1,2,3 . . .), so that the three thresholds are estimated concurrently. This also allows the polarizing current to be set to a defined fraction of the control threshold current on a trial-by-trial basis. Second, the conditioning–test interval can be stepped over a range of values, to track the time course of the changes in excitability induced by the polarizing current. This more elaborate method of tracking the effects of long current pulses on nerve excitability is called threshold electrotonus, and is described below (see Fig. 8). The same principal of tracking several thresholds concurrently and

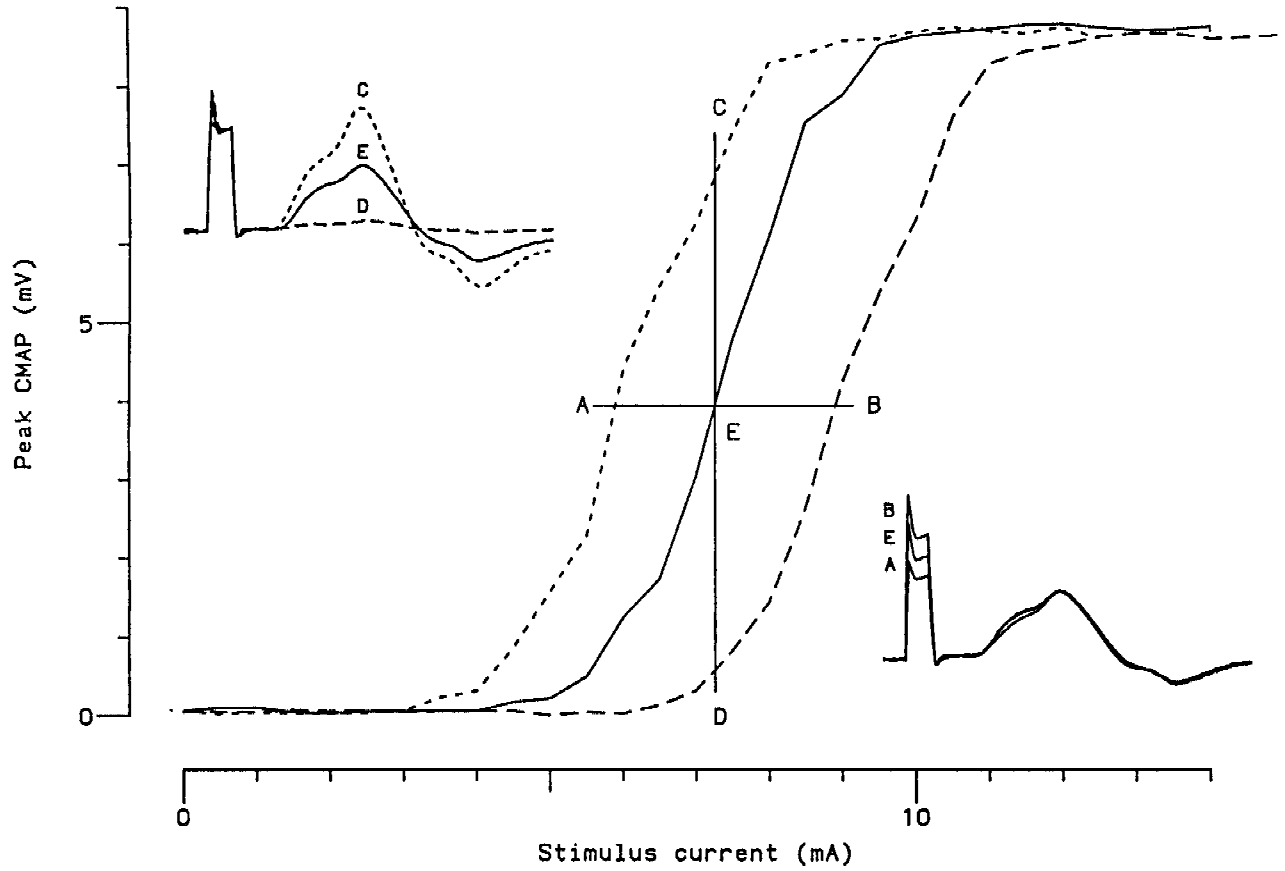

FIGURE 3. Comparison between constant stimulus and constant response measures of nerve excitability. Main curves: stimulus–response characteristics of same nerve-CMAP preparation as in Figures 1 and 2: control (continuous line), with 10% depolarizing current (short-dashed line), and with 10% hyperpolarizing current (long-dashed line). Inset top left: three responses with constant stimulus. Inset bottom right: three responses with threshold tracking. Inset scaling: vertical 1/5th main plot, horizontal sweep = 25 ms. E: control; A, C: with 10% depolarizing current; B, D: with 10% hyperpolarizing current.

stepping one parameter through a range of values can be applied to most of the threshold tracking techniques. For example, seven thresholds were tracked concurrently for the latent addition study in Figure 6. Alternatively, threshold(s) can be tracked while the nerve environment is changed, e.g., by ischemia, hyperventilation, or applying drugs. Skin temperature should be maintained at 32–34°C close to the stimulation site, since ion channel and pump kinetics are strongly temperature-dependent.

Equipment. The main requirements for threshold tracking are: (1) conventional EMG electrodes and preamplifier for recording nerve or muscle action potentials; (2) a remote-controlled, isolated, constant-current stimulator and (especially for threshold electrotonus) nonpolarizable electrodes; and (3) a threshold tracker, which is now more likely to be a computer than a hard-wired circuit. So far as we are aware, there is no suitable isolated stimulator available commercially. Most of the recordings illustrated here (Figs. 2, 3, 5–12) were made with purpose-built stimulators, controlled by a personal computer running the program QTRAC (Copyright: Institute of Neurology). This program incorporates a number of features which are essential and others that are desirable for threshold tracking experiments, including: (a) a choice of tracking modes for determining the step size, from fixed step (e.g., 2%) to proportional step (i.e., step size proportional to difference between actual and target response, as in Figure 2 right); (b) the facility to cycle between a number of stimulus channels, each with different conditioning + test stimulus combinations, keeping separate records of the threshold and tracking history for each; (c) the option to set the amplitude of the conditioning stimulus on one channel to a specified fraction of the test stimulus on another (used for latent addition and threshold electrotonus); (d) the option to subtract the response on one channel from that on another, before comparing the response with the target response (used for impulse-dependent thresh-

old changes, where the response to the conditioning stimulus overlaps with the response to the test stimulus); and (e) the option to increment one of a number of time intervals (e.g., conditioning–test interval), either in regular steps or in a predetermined irregular sequence, and either after a fixed number of test stimuli or only when threshold has been determined to a specified accuracy.

Constant Stimulus vs. Constant Response Measures of Excitability. Since excitability changes can be measured instantaneously by applying a constant submaximal stimulus and recording the changes in nerve or muscle response, it might be wondered whether the extra equipment and time required for threshold tracking was justified. The two methods are compared in Figure 3 for the experiment in Figure 2. The control stimulus–response curve is the continuous line through E, the point taken to define resting threshold and target response. Applying the 10% depolarizing current shifted the stimulus–response curve to the left (short-dashed line AC). This change in excitability could be shown immediately by the change in response from E to C (Fig. 2 right and top left inset in Fig. 3) using a constant stimulus. Alternatively, the change in excitability was shown a few seconds later by the change in stimulus from E to A (Fig. 2 right and bottom right in Fig. 3) required to evoke the same response. Likewise, hyperpolarization caused a decrease in excitability shown by the altered response D or the altered stimulus B. Clearly both methods detect a change in excitability and the constant stimulus method is simpler, quicker, and more sensitive. However, the constant response method, threshold tracking, has advantages which usually outweigh the disadvantages: (1) the dynamic range is greater, enabling threshold increases of 200% or more to be tracked (Fig. 9B), while in Figure 3 threshold increases of more than 20% reduce the constant stimulus response to zero; (2) the response waveforms in Figure 3 (bottom right inset) are almost identical, indicating that the same nerve fibers are being excited; the changes in excitability being measured are therefore a property of those axons, and effectively a property of the ''target axon'' which was last recruited into the target response; (3) conversely, the change in response measured by the constant stimulus method depends not only on the properties of the axons, but also on the shape of the stimulus–response relationship (i.e., on the fiber distribution in the nerve, which is different for motor and sensory axons), and particularly on the level of the control stimulus; and (4) because the threshold changes are properties of a single ''target axon,'' they may, when normalized with respect to the resting value, be compared with threshold changes in a single fiber or different type of nerve preparation, or even with a computer model of a nerve fiber. The threshold tracking method is also preferable when the latency of the test potential is important; the latencies of responses A and B in Figure 3 can be compared more readily than those of C and D.

Single Unit vs. Multiunit Thresholds. Threshold tracking can also be applied to single motor units[8,15,18] (e.g., Fig. 4A). It takes longer to determine a single-unit threshold accurately, because of spontaneous fluctuations over a range of as much as 10%.[8,25] Compound nerve and muscle action potentials are more convenient for clinical use, and are used in almost all the studies reviewed here. Threshold tracking seems to work best when either many units or only one contribute to the target response. When there are a few units, and especially when they are abnormal (e.g., in motor neuron disease), then the unit with the lowest unconditioned threshold may not have the lowest conditioned threshold, so that the threshold changes do not reflect the property of a particular fiber. It is important to take account of possible nerve fiber inhomogeneity when interpreting compound action potential thresholds.

UNCONDITIONED THRESHOLD MEASUREMENTS

From the instrumental point of view, the simplest type of threshold tracking experiment is one in which only regular test stimuli are delivered, and a change in threshold follows a change in the nerve environment. This may be brought about by ischemia, by hyperventilation, or by injection of an anesthetic or other drug. This requires only a single channel threshold tracker, as was used in the first application of automatic threshold tracking to human nerves by Grafe and colleagues.[30,88]

Ischemia and Ischemic Resistance. Substantial changes in membrane potential are induced in human axons in vivo by applying a pneumatic tourniquet to a limb and making the nerve under and distal to the cuff ischemic. Provided the cuff is only inflated for a few minutes, the procedure is painless and the nerves recover normal excitability within an hour (Fig. 4). Ischemia inhibits the sodium pump (Na^+/K^+–adenosine triphosphatase) and causes membrane depolarization. (This is partly a direct effect, because the pump is electrogenic, and partly indirect, due to the subsequent extracellular accumulation of potassium ions and the decline of the potassium equilibrium potential.[8]) On release of

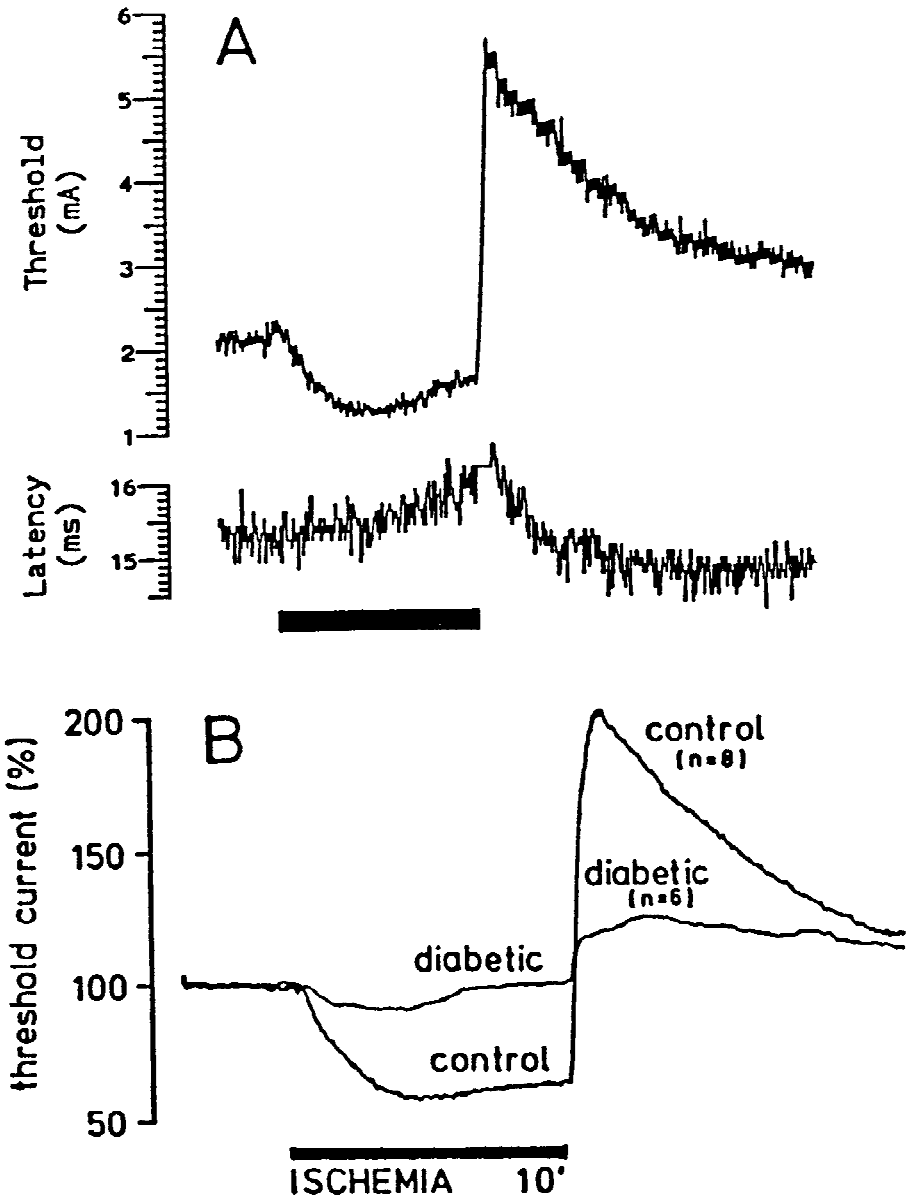

FIGURE 4. Excitability changes during ischemia measured by threshold tracking. **(A)** Threshold changes in single motor axon induced by 10 min of ischemia (black bar), and response latencies recorded when unit excited. Motor unit in first dorsal interosseous was isolated with intramuscular electrode. Ulnar nerve was stimulated at elbow with 1-ms pulses. Ischemia was induced by inflating pressure cuff on upper arm to 200 mmHg. [Replotted from Bostock H, Baker M, Grafe P, Reid G: Changes in excitability and accommodation of human motor axons following brief periods of ischaemia. *J Physiol (Lond)* 1991;441:513–535.] **(B)** Ischemic resistance in diabetes. Averaged, normalized changes in threshold current in 8 controls and 6 patients with diabetes mellitus are shown. Peroneal nerve was stimulated at fibular head; 0.2-ms pulses were adjusted to maintain constant amplitude of CMAP of extensor digitorum brevis. Ischemia was induced by inflating cuff at thigh, 40–50 mmHg above systolic blood pressure. (Reprinted from Weigl P, Bostock H, Franz P, Martius P, Müller W, Grafe P: Threshold tracking provides a rapid indication of ischaemic resistance in motor axons of diabetic subjects. *Electroencephalogr Clin Neurophysiol* 1989;73:369–371 with kind permission from Elsevier Science Ireland Ltd., Bay 15K, Shannon Industrial Estate, Co. Clare, Ireland.)

ischemia the axons rapidly become hyperpolarized, due to hyperactivity of the electrogenic sodium pump. The changes in membrane potential in motor axons during and after ischemia can be followed conveniently by threshold tracking, and similar results are obtained by tracking a constant fraction of the compound muscle action potential as by tracking the threshold of a single fiber.[8,16,30] The extent of the threshold changes is greatest in the proximal parts of the longest motor axons,[16,50] and greater if

the cuff is inflated over, rather than proximal to the site of stimulation.[16] A comparison between the threshold and latency changes in Figure 4A is instructive. During ischemia latency was prolonged by 1 ms, as depolarization inactivated sodium channels,[58] and on release of ischemia latency stayed prolonged as the axons hyperpolarized, although threshold increased rapidly by over 200%. Qualitatively similar observations have been made on sensory fibers.[19,55] Latency therefore increases both with depolarization and with hyperpolarization and does not accurately reflect the changes in excitability.[24] This example also shows that slow conduction does not necessarily imply a disorder of myelination.

The first clinical study with automatic threshold tracking investigated the resistance to ischemia that is common in the nerves of diabetics[88] (Fig. 4B). A marked divergence between controls and diabetics was evident within 5 min of arterial occlusion, and the differences in postischemic hyperpolarization were even more significant.[79] It was later found that most of the variation in the responses to ischemia could be explained by their relationship to mean blood glucose over the preceding 24 h.[79] These clinical observations led to the development of an interesting in vitro rat model of diabetic neuropathy, in which hyperglycemic sensory axons paradoxically exhibited both resistance to anoxia and irreversible damage by anoxia.[71,80]

Threshold tracking studies in normal subjects have been helpful in revealing the mechanism of the postischemic ectopic discharges that arise in motor axons after extended periods of ischemia.[16,17] They have also been used to investigate the mechanism of postischemic paresthesias, by comparing the responses of motor fibers and cutaneous afferents to ischemia.[19] The biophysical mechanisms implicated are likely to be relevant to some types of pathological discharge, particularly those associated with nerve injury, but fall outside the scope of this review. However, the comparison of ischemic and postischemic threshold changes in motor and sensory axons has recently been extended to patients with amyotrophic lateral sclerosis (ALS), to follow up reports that patients with ALS, like diabetics, are resistant to ischemia and experience less paresthesias.[63,75] Threshold tracking has confirmed some abnormalities in the responses of the ALS patients to ischemia, but they are qualitatively and quantitatively very different from those in the diabetic patients (Mogyoros I, Kiernan MC, Burke D, Bostock H: Ischemic resistance of cutaneous afferents and motor axons of patients with amyotrophic lateral sclerosis; submitted for publication, 1997).

THRESHOLD CHANGES FOLLOWING A SINGLE IMPULSE

Following an impulse, myelinated axons go through an oscillating sequence of excitability changes, the recovery cycle (Fig. 5): they are initially inexcitable (absolute refractory period), then excitable with a raised threshold (relative refractory or early subnormal period), then after about 3 ms they become more excitable than normal (supernormal or superexcitable period), and this is usually followed by a further period of raised threshold (late subexcitable or late subnormal period).[8,33,82] (The terms subnormal and supernormal are commonly used as equiva-

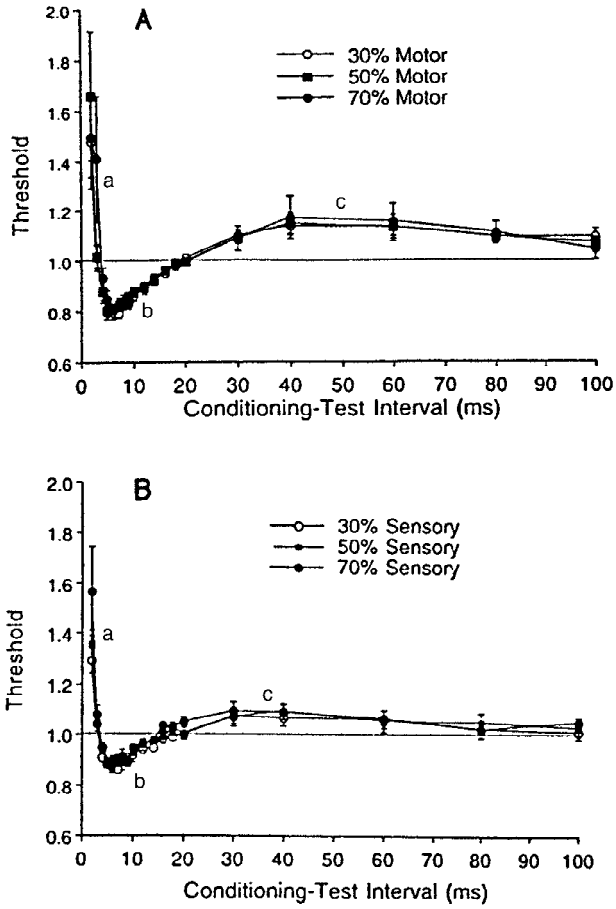

FIGURE 5. Recovery cycles of motor and sensory axons in median nerve at wrist, following single supramaximal conditioning stimulus. Target responses of 30%, 50%, and 70% of the maximum response were tracked in each case. **(A)** motor; **(B)** sensory. Each data point shows mean threshold ± SE for 6 subjects, normalized with respect to the control (unconditioned) stimulus current required for the stated response. a, relative refractory period; b, superexcitable period; c, late subexcitable period. (Reproduced from Kiernan MC, Mogyoros I, Burke D: Differences in the recovery of excitability in sensory and motor axons of human median nerve. *Brain* 1996;119:1099–1105, by permission of Oxford University Press.)

lent to subexcitable and superexcitable, but we suggest that they are best kept for the accompanying changes in latency, which is a more convenient measure for some purposes.[24,81–83]) While the refractoriness is primarily due to sodium channel inactivation, the later excitability changes are primarily due to changes in membrane potential (afterpotentials); thus superexcitability correlates well with the depolarizing afterpotential, due to long-lasting depolarization of the internodal axon, passively depolarized during the action potential, while the late subexcitability correlates with a late hyperpolarizing afterpotential, due to the even slower turn-off of slow potassium channels.[6,7] (A less simplified account of recovery cycle mechanisms will be found in a recent editorial.[82]) All these excitability changes are strongly dependent on membrane potential. Accordingly, changes in these parameters can be used to follow changes in membrane potential, and their absolute values can be used as indirect measures of resting membrane potential, to make comparisons between groups of subjects.

Superexcitability. Superexcitability was first used as a measure of membrane potential in experiments on the effects of trains of impulses on conduction in single demyelinated rat fibers in vivo.[20] The fact that the superexcitability of fibers increased before conduction failed was part of the evidence that hyperpolarization by the sodium pump was responsible. A similar argument was later used to provide evidence that the ischemic resistance of motor axons in diabetic patients (see above, Fig. 4) is not due to membrane depolarization, as had previously been suggested.[79] The close correspondence in the superexcitability, measured at 10, 20, and 30 ms after an impulse, between controls and diabetics made that hypothesis untenable. However, that study did not actually use threshold tracking, but the quicker constant stimulus method (see above), with careful setting of the stimulus level. Measurements of superexcitability by automatic threshold tracking have been used in several recent studies.[19,43–45,54,55,58] Although this use of superexcitability as an index of membrane potential is usually valid, it is important to note that it depends on the voltage dependence of the conductance of the internodal axon membrane,[7] which depends on fast potassium channels. If these are blocked (e.g., by 4-aminopyridine[6]), or if the potassium gradient across the membrane is reversed,[18] then the normal relationship between superexcitability and membrane potential breaks down. For this reason superexcitability can only be trusted as a measure of membrane potential if supported by other measures, such as refractoriness and strength–duration time constant, which depend on sodium rather than potassium channels.

Refractoriness. Refractoriness can be measured as the increase in threshold during the relative refractory period (e.g., at 2 ms after a suprathreshold conditioning stimulus). It depends on membrane potential for reasons different from superexcitability, principally the voltage-dependence of sodium channel inactivation. From a practical point of view, measurements of refractoriness suffer from the disadvantages that (a) temperature must be particularly well controlled; (b) the conditioning stimulus must be supramaximal (and this may be uncomfortable) to ensure that all fibers are made refractory; and (c) the response to the conditioning stimulus alone needs to be subtracted on-line from the response to the conditioning + test pair (a procedure that is also necessary when measuring superexcitability). Nevertheless measurements of refractoriness by threshold tracking are very sensitive to membrane potential, and they have provided a useful additional indication of when changes in membrane potential occur.[44,45,55,58] Of course the refractory period of a CMAP depends not only on the local membrane potential, but also on the ability of the remainder of the nerve, and of the neuromuscular junction, to transmit action potentials faithfully. McDonald and Sears[53] coined the term ''refractory period of transmission'' (RPT) to emphasize this different aspect of refractoriness.[32] Clearly, if the RPT exceeds the interval at which refractoriness is being estimated, the threshold tracking can no longer work, as can occur when tracking the effects of impulse trains on refractoriness of motor axons (Vagg R, Mogyoros I, Kiernan MC, Burke D: Activity-dependent hyperpolarization of motor axons: implications for multifocal motor neuropathy; submitted for publication, 1997). RPT is increased by demyelination, or any other factor that reduces the security of impulse transmission, and since it is determined by the weakest link in the chain, measurements of RPT[2,32] and RPT distributions[38,69] have been found to be more sensitive than conduction velocity measurements in detecting neuropathies. There is a substantial literature on this topic, which falls outside the scope of this review.[47,76]

THRESHOLD CHANGES FOLLOWING TRAINS OF IMPULSES

Bergmans[8] showed that there are two quite different mechanisms causing an increase in threshold after trains of impulses. Hyperpolarization is involved in each case, and he called these mechanisms H1 and

H2, since they correspond to two components of hyperpolarizing (or ''positive'') afterpotential, P1 and P2.[31] H1 is due to activation of slow potassium channels, and can be regarded as a summation of late subexcitability; it saturates after about seven impulses (over a maximum of 40 ms), and lasts about 100 ms.[6,8,85] H2, on the other hand, is due to activation of the electrogenic sodium pump, accumulates over thousands of impulses, and has much slower kinetics, reflecting intracellular sodium accumulation and recovery.[8,18,20,44,45] To these hyperpolarizing effects of impulse trains must be added an opposite, excitatory action, seen only after long, high-frequency trains, and which leads to posttetanic repetitive activity and ectopic discharges.[9] Two aspects of these activity-dependent excitability changes have received attention recently; one is the possible contribution that H1 and H2 may make to conduction failure in neuropathy, and the other concerns the mechanism of the paradoxical hyperexcitability and spontaneous discharges with long trains.

H1 has been investigated in normal cutaneous afferents[54,85] and in carpal tunnel syndrome[55] (CTS), but no evidence of increased conduction failure was found, perhaps because the excitability changes produced by H1 are modest. A study of H2 in CTS[44] produced similarly negative findings, once allowance had been made for temporal dispersion, and this was more surprising: the pathologically slowed axons in CTS did not behave as expected for demyelinated axons with a marginal safety factor,[20] and the authors questioned whether demyelination could be the cause of the conduction slowing and resting conduction block. Impulse trains sufficient to activate H2, such as were used in this CTS study,[44] will never have widespread clinical application, because the stimulus trains are painful. However, it has recently been shown that H2 can be measured by threshold tracking following voluntary activation of motor fibers, rather than tetanic stimulation (Vagg R, Mogyoros I, Kiernan MC, Burke D: Activity-dependent hyperpolarization of motor axons: implications for multifocal motor neuropathy; submitted for publication, 1997). This may prove a more widely acceptable way of monitoring sodium pump activity, and could be used to test this function (e.g., in diabetics); the time taken to remove an activity-dependent sodium load could provide a good index of pump activity.

The mechanism of the paradoxical posttetanic hyperexcitability following long, high-frequency stimulation is of interest in providing a possible model for neuropathic discharges, such as in neuromyotonia.[10] This phenomenon was recently studied in some detail in a single motor axon with the help of threshold tracking[18]; it was attributed to a buildup of extracellular potassium ions, and linked to similar phenomena induced in rat axons by injecting potassium ions into or under a myelin sheath.[28,42] In both situations axonal membrane potential becomes unstable because the electrochemical gradient for potassium ions across the internodal axolemma is reversed; current through potassium channels moves into the axon, causing depolarization, thereby opening more potassium channels and increasing further the inward potassium current, and so on. The posttetanic ectopic activity in cutaneous afferents responsible for posttetanic paresthesias[45,46] probably has a similar mechanism, the differences in behaviour of tetanized motor axons and cutaneous afferents being attributable to differences in inward rectification and persistent sodium conductance (see below). Inward potassium currents may also underlie some types of neuropathic discharge, but this has not yet been demonstrated.

THRESHOLD CHANGES INDUCED BY SUBTHRESHOLD CURRENTS

Depending on the duration of applied currents, very different types of information can be obtained. Current pulses lasting several minutes have been used in animal experiments to produce a steady-state polarization of the nerve,[20] but safety considerations severely restrict the clinical use of such direct current (DC) polarizing currents, and they will not be considered further. Very brief (submillisecond) pulses produce changes in membrane potential confined to the node of Ranvier and myelin sheath. These changes in potential add to the changes in potential induced by a subsequent test stimulus, provided it comes before the effects of the first have died away. Tasaki[84] referred to this phenomenon as latent addition. Because the factors affecting latent addition also determine the strength–duration behavior of the axons, strength–duration properties will also be considered in this section. When the applied currents last longer than a few milliseconds, sufficient current penetrates the myelin sheath to alter the potential difference across internodal axon membrane, and the net changes in membrane potential (electrotonus) become quite complicated, because they depend on a variety of ion channels, some nodal and some internodal.[6,13] The changes in threshold associated with electrotonus are known as threshold electrotonus.[15]

Latent Addition. The first observations on latent addition in human axons were made by Panizza et al.,[61] using manual determination of the threshold

changes produced by 50-μs depolarizing pulses, set to 50 or 90% of threshold. They estimated time constants averaging 318 μs for sensory and 137 μs for motor fibers, similar to their previous estimates for the strength–duration time constants.[60] Bostock and Rothwell[21] have recently reinvestigated latent addition with automatic threshold tracking, using 60-μs pulses of six amplitudes, ranging from 90% of threshold (depolarizing) to −90% (hyperpolarizing), to evaluate the role of voltage-dependent conductances. Recovery from depolarizing pulses was found to be much slower than that from hyperpolarizing pulses, implying an important contribution from the local response of nodal sodium channels to the latent addition (Fig. 6). Moreover, the difference between motor and sensory fibers could not be attributed to a difference in membrane time constant, which was close to 45 μs for both (Fig. 6C, D). Instead it was proposed that the sensory fibers have a greater resting activation of a persistent sodium conductance, which induces an additional slow component in the recovery of threshold from hyperpolarizing pulses (Fig. 6B, D) and prolongs the strength–duration time constant.[21]

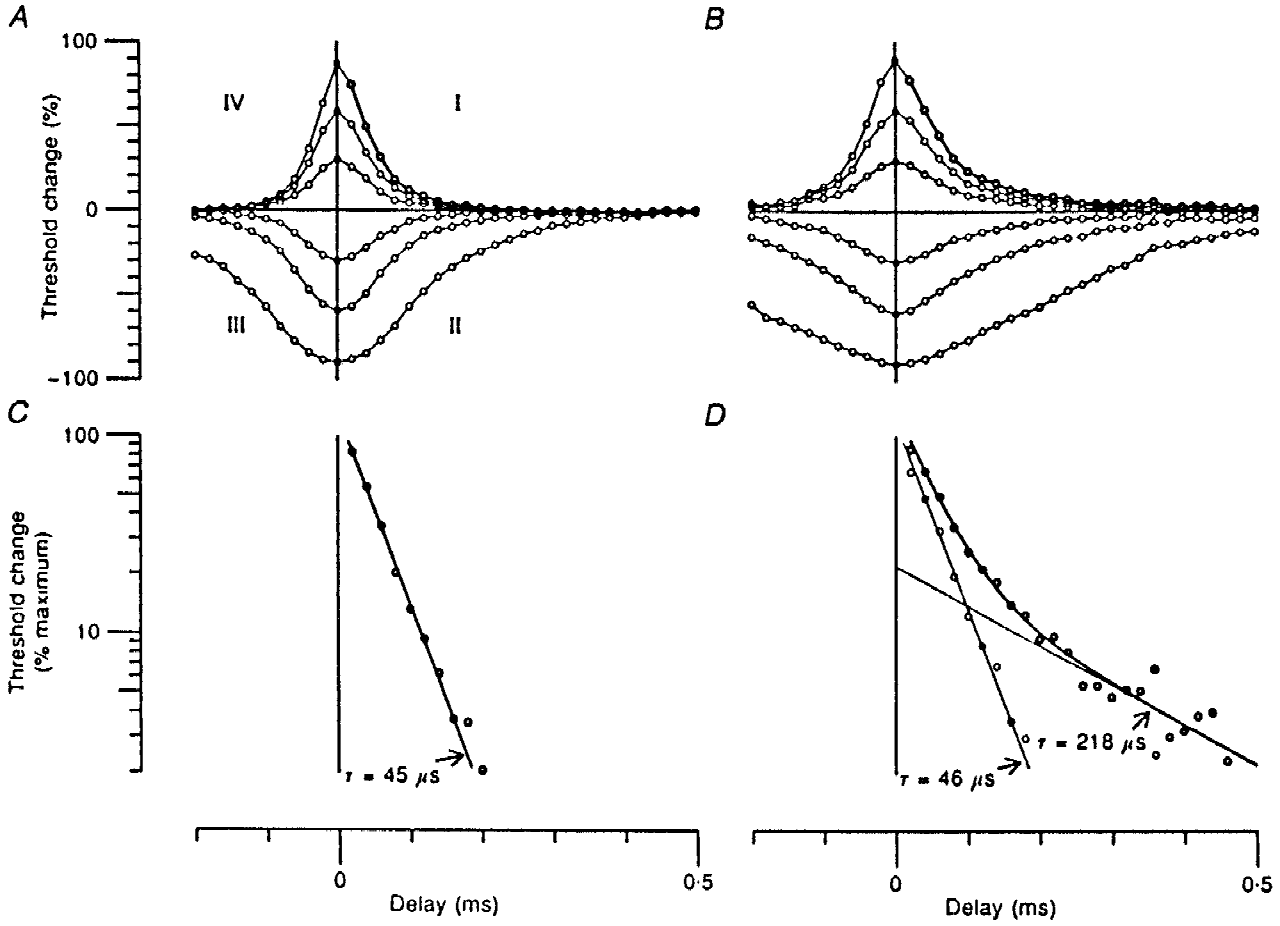

FIGURE 6. Latent addition in motor and sensory axons compared in a normal subject. The ulnar nerve was stimulated at the wrist, and currents were adjusted by threshold tracking to maintain a 40% maximal hypothenar CMAP **(A, C)** or 40% maximal sensory nerve action potential from the little finger **(B, D). (A)** Changes in threshold of motor axons on addition of a brief, subthreshold conditioning current pulse, varying in amplitude from 90% of threshold (bottom curve) to −90% of threshold (top curve) in 30% steps, at conditioning–test delays from −0.2 to 0.5 ms in 20-μs steps. (In quadrants III and IV conditioning pulse follows test pulse.) Conditioning and test stimulus durations were 60 μs. Note slower recovery of excitability following depolarizing (II) than hyperpolarizing (I) conditioning pulses. **(B)** Sensory latent addition, plotted as in **(A).** Note slower recovery from both depolarizing and hyperpolarizing pulses than for motor fibers. **(C, D)** The −90% hyperpolarizing responses in **(A, B)** (quadrant I), replotted on a semilogarithmic scale. Line in **(C)** and curve in **(D)** are best fit single and double exponentials, respectively. In **(D),** the thin lines are the separate fitted fast and slow exponential components, and the open circles are the data points after subtraction of the slow component. Note that the time constant of the fast component of recovery of the sensory fibers (46 μs) is not different from the time constant of the exponential recovery of the motor fibers (45 μs). The fast components were consistent between subjects (mean 43.3 μs) and probably correspond to the passive nodal time constants. [Reproduced from Bostock H, Rothwell JC: Latent addition in motor and sensory fibres of human peripheral nerve. *J Physiol (Lond)* 1997;498:277–294.]

The persistent sodium conductance has now been recorded directly in rat sensory neurons,[5] and is likely to play an important role in many forms of axonal hyperexcitability. Latent addition is at present the best way of assaying this conductance in vivo. For example, one cause of paresthesias in patients is ''hyperventilation syndrome,'' in which respiratory alkalosis causes axonal hyperexcitability. A recent study using threshold tracking to follow changes in latent addition, strength–duration time constant, and other excitability parameters during voluntary hyperventilation has provided evidence that this maneuver produces its effect on nerve excitability by selectively activating the persistent sodium conductance.[58] This interpretation is supported by the high sensitivity of the persistent sodium conductance in rat sensory neurons to block by hydrogen ions.[4]

The technique of latent addition, as applied to human nerves by automatic threshold tracking, is still very new, and no patient studies have yet been reported. It might prove clinically useful in two situations: in conditions of axonal hyperexcitability, to test for abnormal activation of the persistent sodium conductance (as above); or when conduction is slowed or compromised, to test for a change in nodal time constant due to demyelination.

Strength–Duration Behavior. As the duration of a test stimulus is increased, the strength of the current required to just excite a single fiber or a specified fraction of a compound action potential decreases. It has been found by threshold tracking in human peripheral nerve[56] that this strength–duration relationship is remarkably well described by Weiss's[89] empirical law (Glossary and Fig. 7A), just as it is in rat nerves in vivo and in a model myelinated axon.[12,22] When Weiss's law applies, the old term chronaxie is identical to the differently defined strength–duration time constant (see Glossary), and may be estimated reliably from the thresholds for just two pulse durations.[56] As shown earlier,[60] cutaneous afferents have consistently longer strength–duration time constants than motor fibers,[56] most likely because of the more prominent persistent sodium conductance revealed by latent addition.[21] The voltage dependence of this conductance explains why the strength–duration time constant is quite sensitive to membrane potential, being increased by depolarization and reduced by hyperpolarization.[18,21] Because the strength–duration time constant is also related to the passive membrane time constant, it is also increased by demyelination,[12,22] but this has not yet been demonstrated in human nerve.

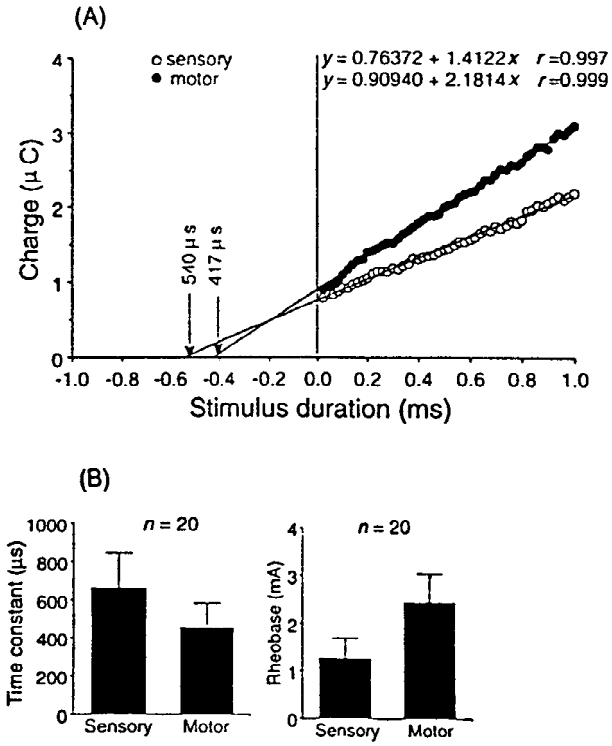

FIGURE 7. Strength duration properties of motor and sensory axons. **(A)** Strength–duration data for motor and sensory axons in median nerve of single subject, plotted as stimulus charge (current × duration) vs. stimulus duration, to show adherence to Weiss's law and method of determining strength–duration time constant (from intercept on duration axis) and rheobase (from slope of regression line). **(B)** Differences between motor and sensory fibers (means ± SDs) in 20 normal subjects (target responses 30% of maximum). The strength–duration time constants of sensory fibers were significantly longer than those of motor fibers ($P < 0.02$) and the rheobases were lower. (Reproduced from Mogyoros I, Kiernan MC, Burke D: Strength-duration properties of human peripheral nerve. *Brain* 1996;119:439–447, by permission of Oxford University Press.)

Measurements of strength–duration time constant can therefore reveal abnormal nodal properties, but on their own they cannot be used to distinguish between abnormalities in resting membrane potential, sodium conductance, or myelination. This technique has recently been applied to two clinical conditions: CTS and ALS. In CTS,[57] strength–duration time constants were normal, whether measured at the site of maximal compression or more proximally, at the wrist. On the other hand, rheobase was abnormally high at both sites. The most likely explanation of this result is that the nodes under the stimulating electrode had such high thresholds that the stimuli preferentially excited normal nodes further away. This example illustrates a limitation of all threshold tracking techniques: they are inappropriate for studying focal neuropathies unless

the stimulus can be confined to the pathological segment, or unless the pathology induces hyperexcitability. In ALS, strength–duration time constants were longer in the motor axons of patients than in age-matched controls (Mogyoros I, Kiernan MC, Burke D, Bostock H: Strength–duration properties of sensory and motor axons in amyotrophic lateral sclerosis; submitted for publication, 1997). An increase in persistent sodium conductance was postulated, since (a) measurements of superexcitability and refractoriness (see above) provided evidence that resting potentials were normal; and (b) rheobases were reduced, which would not be the case if the increase in time constant was due to demyelination. This increase in persistent sodium conductance would be expected to facilitate fasciculations, though inadequate by itself to account for them. Additional biophysical abnormalities in ALS are likely to be involved (see below).

Threshold Electrotonus. In this technique, threshold tracking is used to follow the changes in excitability occurring during and after long-duration (50–500 ms) depolarizing and hyperpolarizing current pulses which are too weak to trigger action potentials. The method is an elaboration of the simple threshold tracking experiment illustrated in Figures 1 and 2. As with latent addition, the conditioning currents are set to defined percentages of the unconditioned threshold current, but the test stimulus duration is usually 1 ms.[15,23,37] With ''proportional tracking,'' it is possible to track the responses to four levels of polarizing current for 200 ms in less than 5 min (Fig. 8), although clinical studies published so far have used slower methods. In contrast to latent addition, threshold changes are conventionally plotted as threshold *reductions*, so that the depolarizing responses are plotted upwards (Fig. 8B), as is normal for electrotonus. By normalizing the threshold

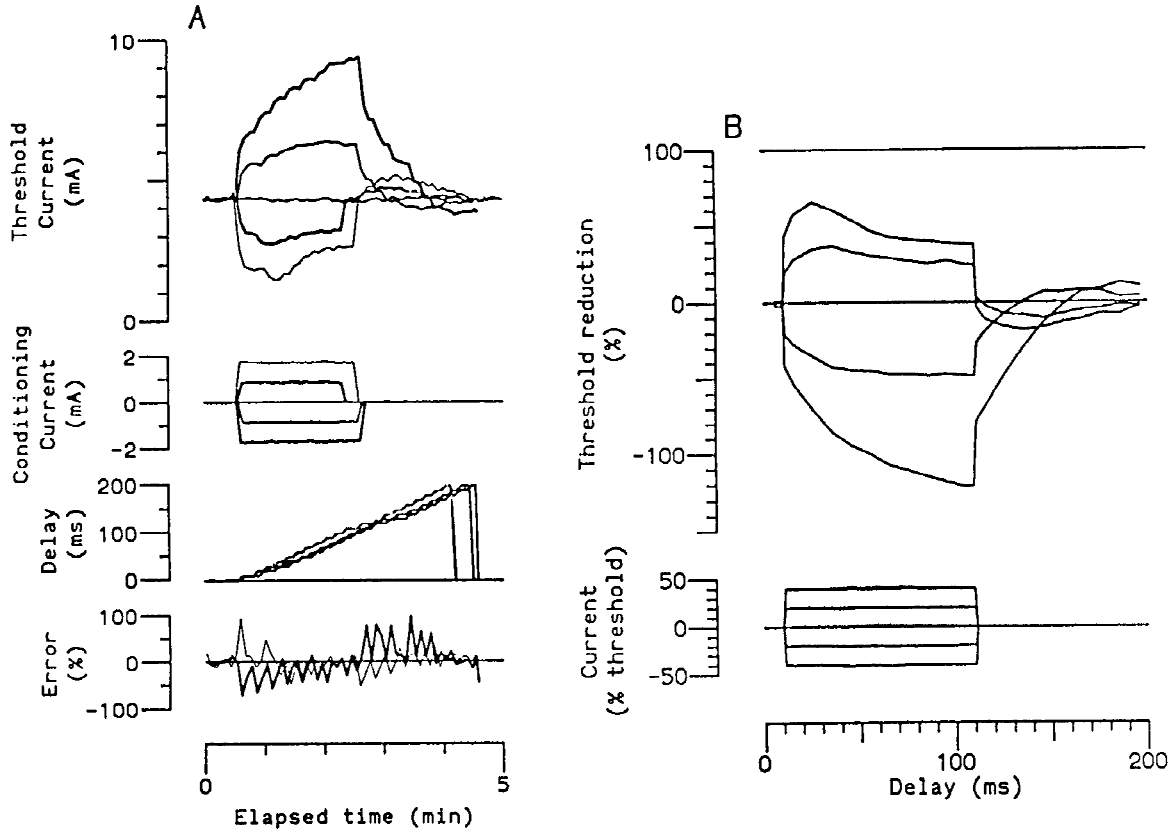

FIGURE 8. Threshold electrotonus recording in normal subject (stimulus to ulnar nerve at wrist, CMAP recorded from hypothenar muscle): quick method with tracking steps proportional to error. **(A)** Data displayed during recording. Top: threshold currents for five different stimulus conditions, tracked in parallel; second from top: conditioning/polarizing currents, set to 40%, 20%, 0, −20%, and −40% of control threshold current, registered at time test stimulus applied; second from bottom: delay between start of conditioning and test pulses, incremented in 10-ms steps; bottom: percentage error in response, as in Figure, 2, plotted for ± 40% conditioning pulses only. **(B)** Threshold electrotonus data replotted from **(A)** against conditioning–test delay (with 10-ms offset). Top: threshold changes plotted as percentage reductions; bottom: conditioning currents, plotted as percentages of control threshold current.

changes with respect to the control threshold, the variation between normal subject becomes quite small, especially for the responses to depolarization (Fig. 9A). Threshold electrotonus waveforms in motor and sensory axons are compared in Figure 9B; the depolarizing responses are surprisingly similar, but differences appear with hyperpolarization and become more marked the longer and stronger the hyperpolarization. The following interpretation of the major components of these threshold electrotonus waveforms is based in part on comparison with recordings of electrotonus in rat spinal roots,[6] in part on recordings of threshold electrotonus in the same preparation,[3,15] and in part on comparison with computer simulations of threshold electrotonus in a simplified model myelinated axon.[17,19,23] (The labels in Figure 9 correspond to the labels previously given for the corresponding components of electrotonus[6,13]).

At the onset and offset of the current, there is a fast change in threshold (F), due to the rapid depolarization or hyperpolarization of the nodes of Ranvier. This is followed by a slower change of threshold in the same direction (S1), as the potential difference across the internodal axon membrane changes more slowly, causing an additional change in potential of the nodal membrane. After about 20 ms, the decrease in threshold on depolarization (S1) is counteracted by a second slow component (S2), which brings threshold back closer to control. S2 is due to activation of slow potassium channels, mainly located at the nodes, which increase threshold partly by hyperpolarizing and partly by reducing the nodal resistance. (These channels are also responsible for the late subexcitability and for H1, and can be blocked with tetraethylammonium ions). For the −80% hyperpolarizing response in Figure 9B, S1 is larger, because hyperpolarization deactivates potassium channels in the internodal axon, and goes on longer, until counteracted by the third slow component (S3). S3 is due to the activation of the axonal inward rectifier, a channel activated slowly by hyperpolarization, and permeable to sodium as well as potassium ions, so that it has an excitatory action. (The inwardly rectifying current is now conventionally designated I_H[62] and is widespread in excitable cells,

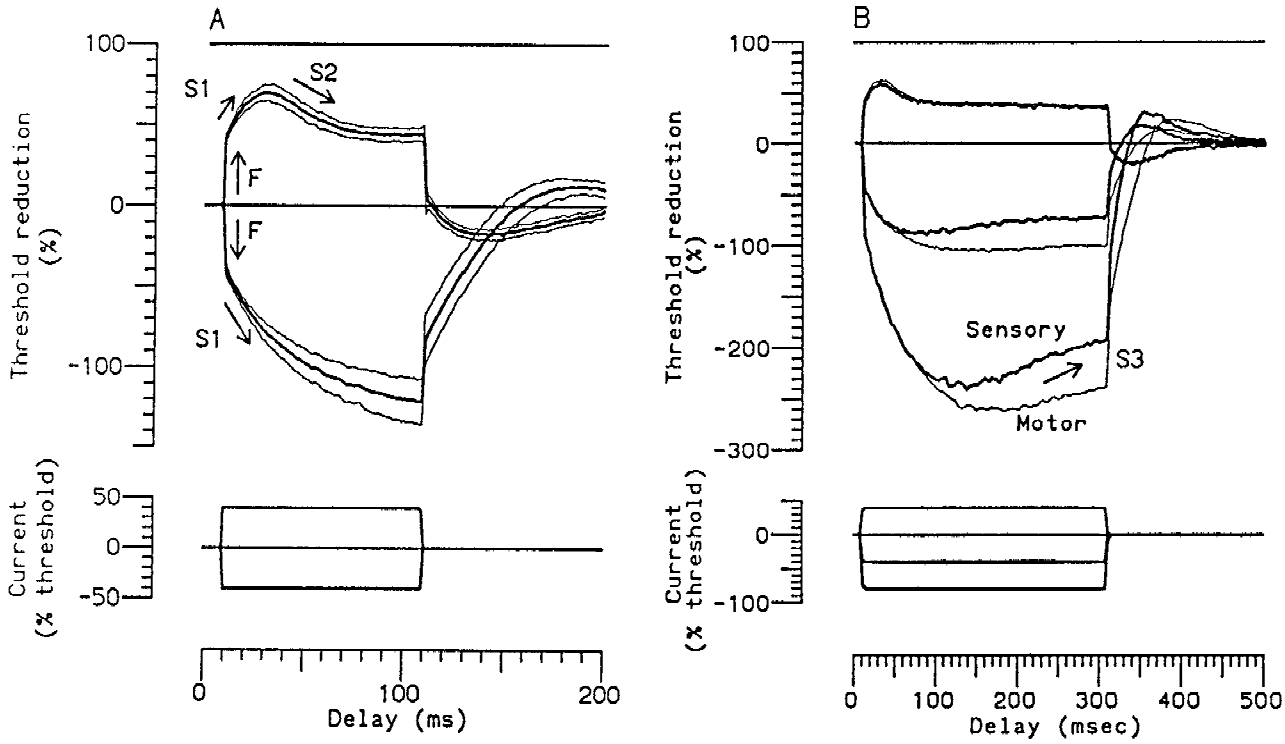

FIGURE 9. Normal threshold electrotonus waveforms and components. **(A)** Averaged motor responses to 100-ms polarizing currents (± 40% of threshold) from 38 subjects, showing fast (F) and slow (S1, S2) components (see text) and small intersubject variability (thick lines: mean, thin lines: mean ± SD). Stimuli were applied to ulnar nerve at wrist, CMAP recorded from hypothenar muscle. (Cikurel K, Murray NMF, Bostock H: unpublished observations, 1997.) **(B)** Comparison between mean motor and sensory responses to 300-ms current pulses (± 40%, −80% of threshold) from 8 subjects, showing additional component of threshold electrotonus (S3), due to inward rectification activated by hyperpolarization. (Reproduced from Bostock H, Burke D, Hales JP: Differences in behaviour of sensory and motor axons following release of ischaemia. *Brain* 1994;117:225–234, by permission of Oxford University Press.)

e.g., it is the basis of cardiac rhythmicity.) Figure 9B provides evidence for a difference in expression of this inward rectifier between motor and sensory axons, which helps to explain their different behavior on release of ischemia[19] and on the cessation of prolonged tetanization.[45,46] Threshold electrotonus waveforms normally correspond reasonably closely to the changes in membrane potential (electrotonus). This close relationship breaks down, however, if the axons become so depolarized that sodium channel inactivation becomes a major determinant of electrical excitability.[3] This occurs during prolonged ischemia, and in a few subjects with ALS (see below).

Threshold Electrotonus as an Indicator of Membrane Potential. As with many other excitability measures, the most important factor determining threshold electrotonus is membrane potential, so threshold electrotonus can also be used as an index of membrane potential. For example, confirmation that the ischemic fall and postischemic rise in threshold (Fig. 4) are primarily due to depolarization and hyperpolarization respectively is provided in Figure 10, which compares changes in threshold electrotonus during and after ischemia with those previously caused by applying DC polarizing currents. Ischemia and depolarization cause a similar ''fanning in'' of the electrotonus waveforms, primarily due to the increased activation of fast and slow potassium channels, while the opposite occurs after release of ischemia or during hyperpolarization. Threshold electrotonus thus provides a very sensitive indicator of changes in membrane potential, and tracking a point on the threshold electrotonus response (e.g., 100 ms after the start of a hyperpolarizing current) is more sensitive than simple threshold tracking at revealing an altered response to ischemia. Quasthoff et al.[65] have used this method to demonstrate that the resistance to ischemia in diabetics (see above) is abolished by several days of antidiabetic treatment. They also found that cancer patients treated with taxol-cisplatin become more sensitive to ischemia.[64]

Threshold Electrotonus and ALS. The first clinical application of threshold electrotonus was to ALS, to see if it could provide evidence of abnormal membrane properties responsible for fasciculations.[23] Motor nerves in 11 patients with definite ALS were compared with 15 normal controls, 6 patients with benign fasciculations, and 25 neurological controls with diseases affecting the tested nerve (ulnar). The neurological and normal controls gave similar responses, but those of the ALS patients were more

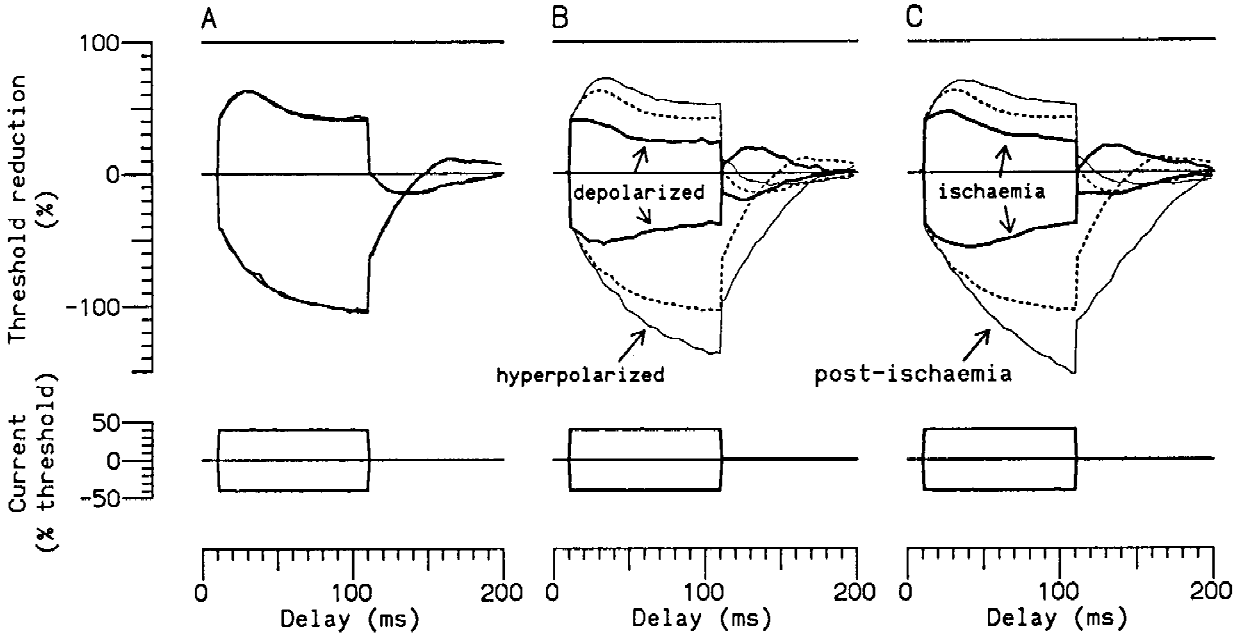

FIGURE 10. Effects on threshold electrotonus of changes in membrane potential induced by polarizing currents and ischemia. **(A)** Control recordings of threshold electrotonus in motor axons to ± 40% conditioning currents (same subject and recording conditions as in Figure, 8), before and after making polarized recordings in **(B). (B)** Recordings in same nerve, while applying DC polarizing currents, set to 20% (thick lines) and −10% (thin lines) of control threshold current. Dotted lines are means of control recordings in **(A). (C)** Recordings in same nerve 5–9 min after inflating pressure cuff on upper arm to induce ischemia (thick lines), and 1–6 min, after releasing the pressure cuff (thin lines). Dotted lines are means of control recordings in **(A).**

variable (Fig. 11). In 7 patients depolarization reduced threshold more than normal (''Type 1,'' e.g., Fig. 11A), but in 4 the threshold increased sharply (''Type 2,'' e.g., Fig. 11B, C). It was suggested that an imbalance between sodium and potassium currents led to abnormal depolarization, which in the Type 2 cases was so extreme as to lead to a threshold increase because of sodium channel inactivation. A similar threshold electrotonus abnormality could be induced in rat nerves by blocking potassium channels,[23] but the change in strength–duration behavior found in a later study (see above) suggests that this was not the only reason for the abnormal responses. Another group of 11 ALS patients tested in Japan showed similar abnormalities.[48]

In a third series of 27 ALS patients[37] (testing the median rather than the ulnar nerve), many responses fell within the normal range, but the average showed significant fanning out (Fig. 12B), more similar to the effects of hyperpolarization (Fig. 10) than the original ''Type 1'' responses (Fig. 11A). The fanning out on hyperpolarization is due to the deactivation of potassium channels, so that these results are compatible with the proposal that potassium conductances are reduced in ALS. A fourth series of 70 ALS patients has given more equivocal results.[27] The depolarizing responses of the patients were abnormally variable, exhibiting other distinctive abnormalities in addition to the Type 1 and Type 2 in Figure 11, but the mean responses were not significantly different from controls. When divided into subgroups, only the ''definite ALS'' and ''progressive muscular atrophy'' groups (showing signs of lower motor neuron involvement) exhibited the abnormalities in threshold electrotonus, whereas the ''bulbar'' and ''primary lateral sclerosis'' groups were normal. No correlation was found between threshold electrotonus and disease progression or CMAP amplitude; in 1 case when only a single hypothenar motor unit was left, threshold electrotonus

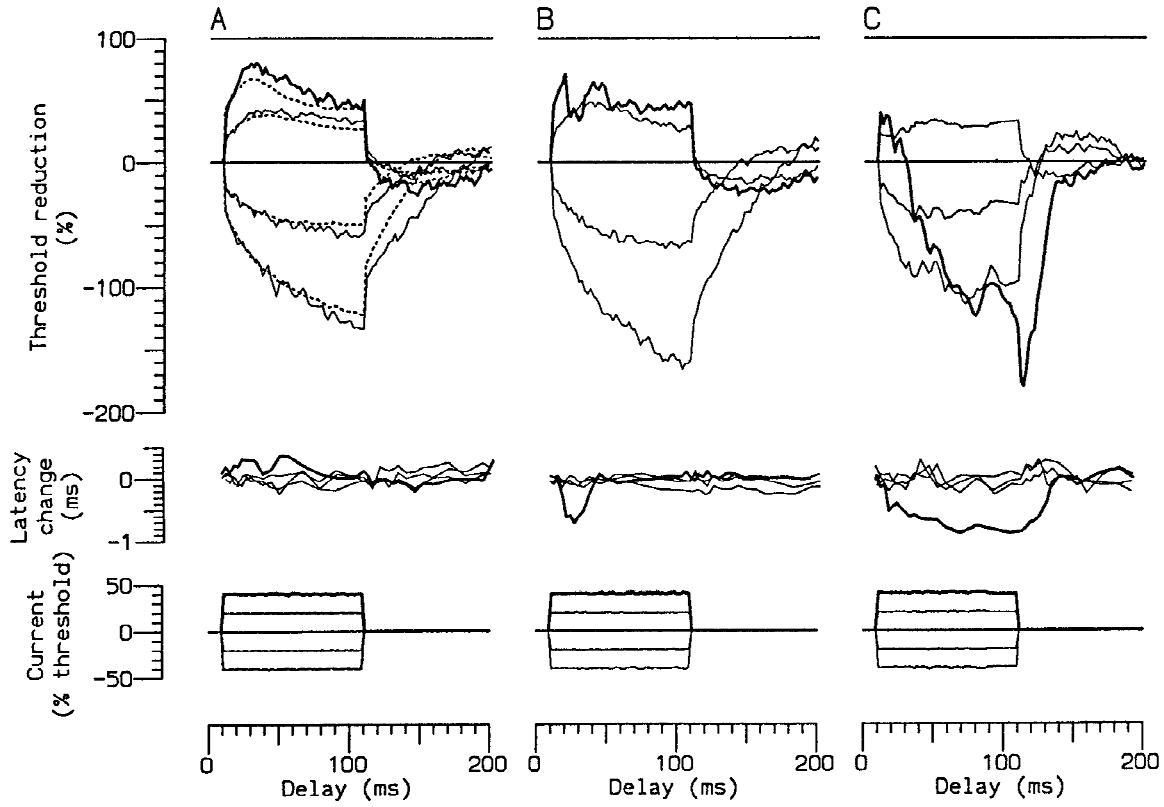

FIGURE 11. Different types of abnormal threshold electrotonus waveforms, recorded from 3 patients with definite ALS. Stimulus was applied to ulnar nerve at wrist, CMAP recorded from hypothenar. Responses to depolarizing currents, 40% of threshold, are plotted with thick lines. **(A)** ''Type 1'' response (continuous lines), with abnormal threshold increase on depolarization, compared with mean of 12 normal controls (broken lines), shown by 6/11 ALS patients. **(B, C)** ''Type 2'' responses with abrupt or profound increases in threshold, shown by 4/11 patients. Center row: difference in latency between responses occurring during the polarizing currents and the responses to control threshold stimuli, showing reduced latency associated with ''Type 2'' responses, evidence of abnormal depolarization. (Reprinted from Bostock H, Sharief MK, Reid G, Murray NMF: Axonal ion channel dysfunction in amyotrophic lateral sclerosis. *Brain* 1995;118:217–225, by permission of Oxford University Press.)

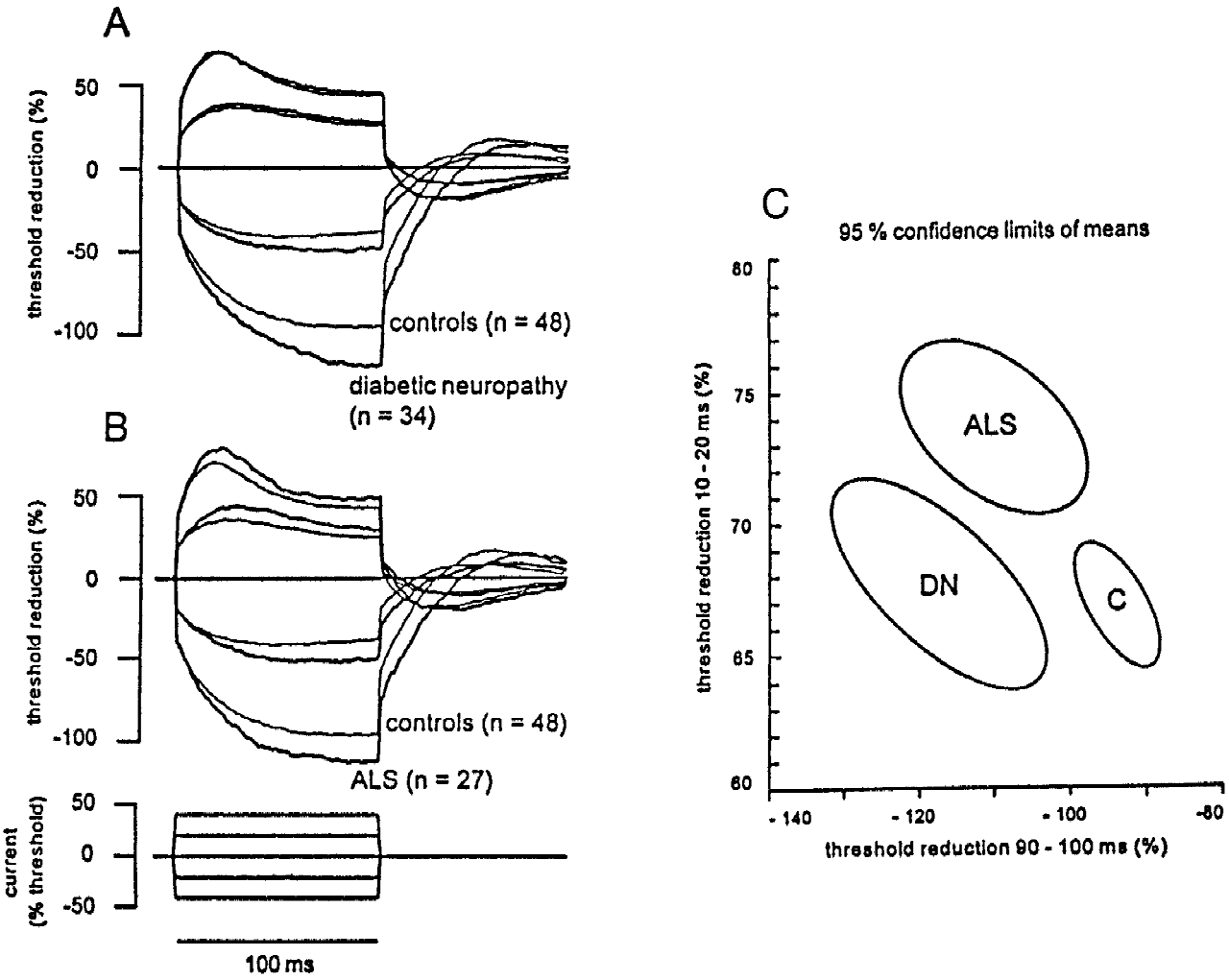

FIGURE 12. Comparison between threshold electrotonus in diabetic neuropathy and ALS. Stimuli were applied to median nerve at wrist, CMAP recorded from thenar eminence. **(A)** Mean responses from 34 patients with diabetic neuropathy compared with those from 48 controls. **(B)** Mean responses from 27 patients with definite ALS compared with same controls. **(C)** Comparison between the three groups in respect to threshold reduction 90–100 ms after start of −40% hyperpolarizing current (abscissa) and 10–20 ms after start of 40% depolarizing current (ordinate). Ellipses correspond to 95% confidence limits for group means (C = controls, DN = diabetic neuropathy, and ALS), estimated on the assumption that the two threshold parameters follow bivariate normal distributions. (Reprinted from Horn S, Quasthoff S, Grafe P, Bostock H, Renner R, Schrank B: Abnormal axonal inward rectification in diabetic neuropathy. *Muscle Nerve* 1996;19:1268–1275.)

was normal, possibly because the surviving motoneuron had not yet succumbed to the disease process. However, since the fasciculations in ALS usually arise in the motor nerve terminals,[51] it is possible that abnormal membrane properties are often confined to that region and inaccessible to threshold electrotonus.[14]

Threshold Electrotonus and Diabetic Neuropathy. Horn et al.[37] have reported the results of an extensive study of threshold electrotonus in diabetic patients. They have looked at motor and sensory axons at the wrist in 63 diabetics, and compared the results with those from 50 normal controls and 27 patients with ALS (see above). They found that although only a minority of patient responses were outside the normal range, there were differences between the group means, which became highly significant when the patients were subdivided on the basis of age or presence of neuropathy. The abnormalities occurred only in the responses to hyperpolarization, and were quite distinct from the abnormalities recorded in ALS patients in the same study (Fig. 12A–C). Motor and sensory nerves in diabetics differed from normal in the same way that normal motor nerves differ from sensory ones, so it was concluded that diabetic neuropathy is associated with a deficit in inward rectification.[37] Since inward rectification

has been found in other cells to depend on the level of intracellular cyclic adenosine monophosphate (cAMP),[1,39] this deficiency may be related to the lack of cAMP reported in diabetic nerves.[40]

One function of axonal inward rectification is to limit hyperpolarization by the sodium pump during long-lasting, high-frequency bursts of impulses,[6] so the lack of inward rectification could be responsible for the impaired vibration perception[86] and fatigability of cutaneous afferents[52] in diabetic patients. The unexpected finding that inward rectification is deficient in myelinated fibers in diabetic neuropathy has led to a study of inward rectification in C fibers (which are often the axons most affected by diabetic neuropathy). Threshold electrotonus applied to C fibers in biopsied human sural nerve in vitro has shown that inward rectification is prominent in these fibers.[35] It is activated by the electrogenic sodium pump at frequencies as low as 1 Hz, and a deficiency in diabetic neuropathy could have serious functional consequences.

Threshold Electrotonus in a Toxic Neuropathy. Antitumor treatment with taxol-cisplatin combination chemotherapy causes a rapidly developing, predominantly large-fiber sensory neuropathy.[26] Threshold electrotonus has been used to investigate this neuropathy in 15 patients.[36,70] There was a marked symmetrical ''fanning in'' or flattening of the threshold electrotonus responses, indicating that the axons were depolarized or the conductance of the internodal axon membrane was increased. As already mentioned, the axons also became more sensitive to ischemia. These disturbances in membrane excitability were detected by threshold electrotonus after only one or two doses of chemotherapy, before any clinical or neurological signs of neuropathy.[70] Taxol also depolarized human sural nerves in vitro.[64]

Threshold Electrotonus in Other Conditions. Threshold electrotonus abnormalities have also been reported in multifocal motor neuropathy with conduction block, where the abnormality was restricted to the site of the lesion,[41] and in monomelic amyotrophy with spinal hemiatrophy.[49] Although a considerable number of patients with demyelinating neuropathies have been tested, including some with marked conduction slowing, no consistent abnormalities in threshold electrotonus have been found (Quasthoff, personal communication). This may seem surprising, since alterations in passive as well as active membrane parameters should affect electrotonus, but the explanation is probably similar to that for the negative findings on strength–duration behavior in CTS[57] (see above), i.e., fibers demyelinated at the point of stimulation will preferentially be excited at adjacent, normal nodes, or other, more normal fibers will be excited in their place.

Threshold Tracking Techniques and Models of Nerve Excitability. A good measure of our understanding of nerve excitability is our ability to model it mathematically in terms of the properties of its constituent components, such as the voltage-dependent ion channels. As far as the generation of action potentials by the node of Ranvier is concerned, there is little mystery; voltage clamp studies of nodal currents have led to a series of models of the ion currents in frog,[29] rat,[73] and most recently, human nodes.[74] These models reproduce the appropriate action potential waveforms quite well, and when coupled to model internodes, reproduce saltatory conduction very well.[34] What this classical approach has not told us, however, is how the internodal ion channels which have been revealed by patch clamping[72,87] interact with the nodal channels and with ion pumps to generate the complex repertoire of axonal responses to impulse activity, ischemia, and polarizing currents. Only models which take account of internodal as well as nodal electrogenic mechanisms, as first done by Blight,[11] can hope to explain many types of pathological nerve activity.

Threshold electrotonus provides more information about more different axonal ion channels than any other noninvasive test, and it has therefore been used to help develop mathematical models of human axons. The first attempts were based on Barrett and Barrett's equivalent circuit for the electrical interaction between nodes and internodes,[7,68] with the simplifying assumption that threshold electrotonus paralleled electrotonus.[17,19,77,78] These models helped towards' an understanding of postischemic ectopic discharges,[17] and to demonstrate a difference in inward rectification between motor and sensory fibers.[19] However, electrotonus does not parallel threshold electrotonus in depolarized axons,[3] and when it came to modeling ''Type 2'' threshold electrotonus in ALS (Fig. 11B, C), the assumption that it does had to be abandoned. Instead, threshold electrotonus was simulated by applying threshold tracking to the model, and this improved procedure was able to reproduce some of the abnormal features of threshold electrotonus in ALS.[23]

In these models, sodium channel parameters were assumed to be the same as described for rat nodes of Ranvier.[73] More recently a limitation of that model was exposed by the experiments on latent addition.[21] The responses of human axons in vivo to

brief current pulses (Fig. 6) were found to be critically dependent on the subthreshold behavior of sodium channels, which was best described by equations derived from human data[74] and the assumption of a small persistent sodium conductance, as already described. Current models of human nerve excitability incorporate persistent as well as transient nodal sodium channels, fast and slow potassium channels, and inward rectification. The model represented by the electrical circuit in Figure 13 can account for normal threshold electrotonus waveforms and the changes in threshold electrotonus and strength–duration behavior when nerves are depolarized or hyperpolarized (Mapes R, Bostock H: unpublished observations, 1997). However, despite the simplifications of this model, it still has more parameters than can be determined with confidence from available experimental data. Thus the two threshold tracking techniques of latent addition and threshold electrotonus have enabled considerable progress toward a more complete description of normal and abnormal human nerve excitability, but we are still some way from the goal of being able to test a nerve in vivo and define any channel abnormalities unambiguously.

CONCLUSIONS

Threshold tracking is a powerful tool for investigating excitable membranes; it is well suited to the study of human peripheral nerves in vivo, but has so far

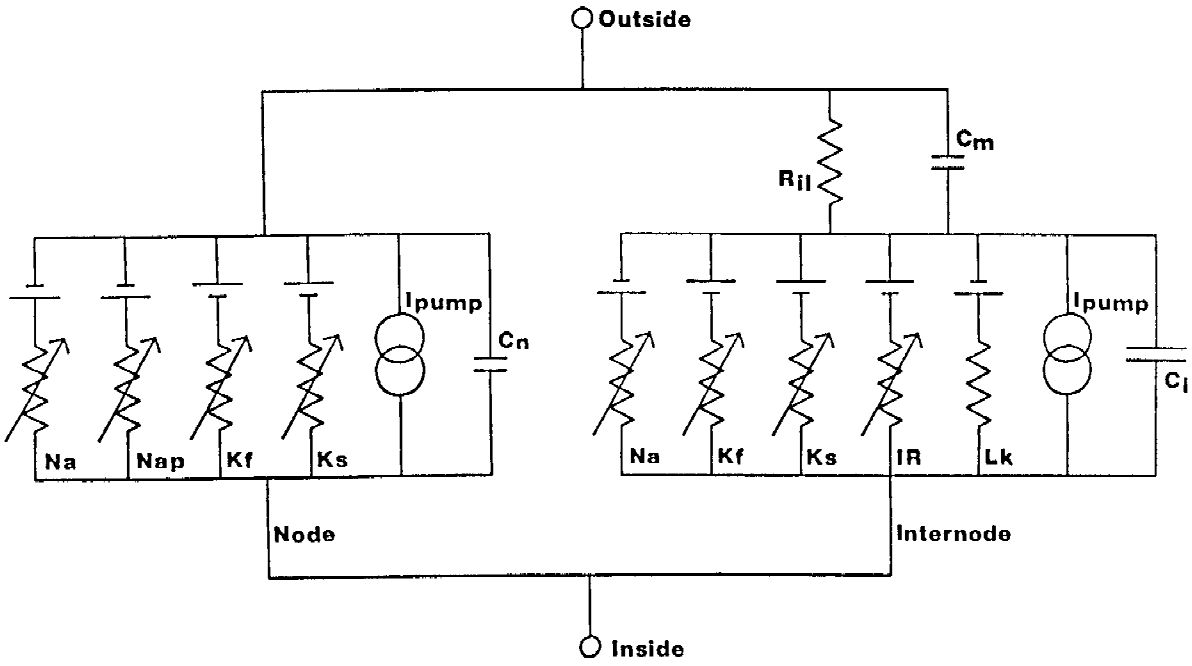

FIGURE 13. Simplified equivalent circuit of node and internode of myelinated axon, showing principal electrical components. A computer model based on this circuit can simulate recovery cycles, latent addition, strength–duration behavior, and threshold electrotonus. Each ion channel type is represented by a cell, with electromotive force corresponding to the Nernst equilibrium potential for the ion, in series with a voltage- and time-dependent conductance. Electrogenic ion pumps (principally Na^+/K^+–adenosine triphosphatase), present in both node and internode, are represented as current sources (I_{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"[7] (R_{il}), which is made up of current paths through and under the myelin (also called the "Barrett–Barrett resistance"[68]). Changes in membrane potential of the internodal axon are slow, because of its high electrical capacitance (C_i), nearly three orders of magnitude greater than the nodal capacitance (C_n). The nodal ion channels illustrated are: Na, sodium channels, responsible for the action potential; Nap, persistent sodium channels,[21] active in subthreshold potential range—they lower rheobase, prolong strength–duration time constant, and facilitate repetitive firing (more prominent in sensory than motor fibers); Kf, fast potassium channels, present but normally of little importance at nodes[74]; and Ks, slow potassium channels, responsible for the late subexcitability, H1, and the S2 component of electrotonus—they reduce excitability both by hyperpolarization and by increasing nodal conductance. The internodal ion channels illustrated are: Na, internodal Na channels—these probably outnumber nodal channels but are not normally activated; they may contribute to some types of pathological discharge[17] and assist conduction in demyelination; Kf, fast potassium channels, present in high concentrations at the paranodes[67]—they limit superexcitability and depolarizing electrotonus; together with slow potassium channels, Ks, they contribute to the maintenance of the resting potential; IR, inward rectifier channels activated by hyperpolarization, responsible for the S3 component of threshold electrotonus (more prominent in sensory fibers); and Lk, leak or non–voltage-dependent channels—they contribute little to electrotonus.

been little exploited clinically. Threshold tracking can be applied to motor or sensory axons to determine changes in excitability caused by a single impulse (e.g., refractoriness, superexcitability), by trains of impulses (H1, H2), by ischemia (e.g., ischemic resistance in diabetes), by changing stimulus duration (e.g., strength–duration time constant), or by subthreshold polarizing currents (latent addition and threshold electrotonus). Excitability changes measured by threshold tracking can be compared between different stimulation sites and different nerves, and with single-fiber preparations and computer models.

All of the methods described provide useful information about membrane properties in normal and/or diseased nerves, but none has yet established a role in the diagnosis or monitoring of a particular clinical condition. We view them primarily as research tools, of greatest value in probing pathophysiological mechanisms. If threshold tracking techniques are to be adopted more widely, it is important to stress the limitations that they have in common. They all test the nerve only at the point of stimulation, and so they are not useful for focal neuropathies, unless it is possible to excite the nerve at the lesion site (as in the case of multifocal motor neuropathy[41]). Second, they test only the axons with thresholds close to the level chosen for tracking, so conditions affecting the excitability of only a minority of axons, whether the least or the most excitable, may go undetected. Third, they take no account of axons that have degenerated, or which are blocked between stimulation and recording site, so that serial measurements can remain unchanged or even show improvements despite a progressive loss of axons (as may occur in ALS). Because of these limitations, threshold tracking may prove of greatest value in metabolic and toxic neuropathies in which axons are affected rather uniformly.

GLOSSARY

Chronaxie. The stimulus duration for which the threshold current is twice the rheobase. Chronaxie is identical to the strength–duration time constant when Weiss's law is obeyed.

Electrotonus. Changes in membrane potential evoked by subthreshold depolarizing or hyperpolarizing current pulses.

H1, H2. Hyperpolarizing aftereffects of trains of impulses, which cause an increase in threshold. They differ in time course and mechanism (see text).

Latent Addition. Addition of the effects of two brief, closely spaced subthreshold current pulses on nerve excitability.

Refractoriness. The decrease in excitability (or increase in threshold) observed during the relative refractory period after a nerve impulse. Refractoriness may be expressed as the percentage change in threshold when a test stimulus is preceded by an exciting stimulus at an interval of e.g. 2 ms. Refractoriness defined in this way is a property of the nerve membrane at the stimulation site, and can only be measured at intervals greater than the refractory period of transmission.

Refractory Period (Absolute). The period immediately after a nerve impulse during which an axon cannot be excited, however great the stimulus.

Refractory Period (Relative). The period between the end of the absolute refractory period and the start of the superexcitable period, when an axon may be excited but its threshold is increased.

Refractory Period of Transmission. The minimum interval between successive suprathreshold stimuli, such that the second impulse successfully reaches the recording site.

Rheobase. The threshold current (or estimated threshold current) for a stimulus of infinitely long duration.

Strength–Duration Time Constant. An apparent membrane time constant inferred from the relationship between threshold current and stimulus duration. It has been defined as the ratio between the minimum charge threshold and the rheobase.[59] In general it is longer than the (passive) membrane time constant. (See Weiss's Law.)

Superexcitability. An increase in excitability (or reduction in threshold current) commonly observed shortly after a nerve impulse. Superexcitability may be expressed as the percentage change in threshold when a test stimulus is preceded by an exciting stimulus at an interval of e.g. 7 ms.

Supernormality. This term is often used interchangeably with superexcitability, but we prefer to use supernormality for the increase in conduction velocity which may accompany superexcitability.

Threshold (Current). The stimulus current required to excite a single axon on 50% of trials, or to evoke a compound nerve or muscle action potential that is a defined fraction of the maximum.

Threshold Electrotonus. Changes in threshold induced by subthreshold current pulses, corresponding to electrotonus (especially changes in threshold to 1-ms current pulses induced by 50–500-ms polarizing currents).

Weiss's Law. An empirical formula for the strength–duration relationship, which is closely followed by myelinated fibers: the stimulus charge [Q, i.e., the product of stimulus current (I) and duration (t)] at threshold is directly proportional to stimulus duration, or $Q = I \cdot t = R\ (t + \tau)$, where R is the rheobase and τ is the chronaxie or strength–duration time constant.

REFERENCES

1. Akasu T, Shoji S: cAMP-dependent inward rectifier current in neurons of the rat suprachiasmatic nucleus. *Pflügers Arch* 1994;429:117–125.
2. Alderson K, Petajan JH: Relative refractory period: a measure to detect early neuropathy in alcoholics. *Muscle Nerve* 1987; 10:323–328.
3. Baker M, Bostock H: Depolarization changes the mechanism of accommodation in rat and human motor axons. *J Physiol (Lond)* 1989;411:545–561.
4. Baker MD, Bostock H: Ca^{2+} and H^+ ion block of persistent Na^+ current in DRG neurones. *Prog Biophys Molec Biol* 1996;65 (suppl 1):112.
5. Baker MD, Bostock H: Low-threshold, persistent sodium current in rat large dorsal root ganglion neurons in culture. *J Neurophysiol* 1997;77:1503–1513.
6. Baker M, Bostock H, Grafe P, Martius P: Function and distribution of three types of rectifying channel in rat spinal root myelinated axons. *J Physiol (Lond)* 1987;383:45–67.
7. Barrett EF, Barrett JN: Intracellular recording from vertebrate myelinated axons: mechanism of depolarizing afterpotential. *J Physiol (Lond)* 1982;323:117–144.
8. Bergmans J: *The Physiology of Single Human Nerve Fibres*. Vander, Belgium, University of Louvain, 1970.
9. Bergmans J: Physiological observations on single human nerve fibres, in Desmedt JE (ed): *New Developments in Electromyography and Clinical Neurophysiology*. Basel, Karger, 1973, vol 2, pp 89–127.
10. Bergmans J: Repetitive activity induced in single human motor axons: a model for pathological activity, in Culp WJ, Ochoa J (eds): *Abnormal Nerves and Muscles as Impulse Generators*. New York, Oxford University Press, 1982, pp 393–418.
11. Blight AR: Computer simulation of action potentials and afterpotentials in mammalian myelinated axons: the case for a lower resistance myelin sheath. *Neuroscience* 1985;15:13–31.
12. Bostock H: The strength-duration relationship for excitation of myelinated nerve: computed dependence on membrane parameters. *J Physiol (Lond)* 1983;341:59–74.
13. Bostock H: Mechanisms of accommodation and adaptation in myelinated axons, in Waxman SG, Stys PK, Kocsis JD (eds): *The Axon*. Oxford, Oxford University Press, 1995, pp 311–327.
14. Bostock H: Abnormal excitability of motor axons in ALS, in Kimura J, Kaji R (eds): *Physiology of ALS and Related Diseases*. Amsterdam, Elsevier, 1997, pp 133–142.
15. Bostock H, Baker M: Evidence for two types of potassium channel in human motor axons in vivo. *Brain Res* 1988;462: 354–358.
16. Bostock H, Baker M, Grafe P, Reid G: Changes in excitability and accommodation of human motor axons following brief periods of ischaemia. *J Physiol (Lond)* 1991;441:513–535.
17. Bostock H, Baker M, Reid G: Changes in excitability of human motor axons underlying post-ischaemic fasciculations: evidence for two stable states. *J Physiol (Lond)* 1991;441: 537–557.
18. Bostock H, Bergmans J: Post-tetanic excitability changes and ectopic discharges in a human motor axon. *Brain* 1994;117: 913–928.
19. Bostock H, Burke D, Hales JP: Differences in behaviour of sensory and motor axons following release of ischaemia. *Brain* 1994;117:225–234.
20. Bostock H, Grafe P: Activity-dependent excitability changes in normal and demyelinated rat spinal root axons. *J Physiol (Lond)* 1985;365:229–257.
21. Bostock H, Rothwell JC: Latent addition in motor and sensory fibres of human peripheral nerve. *J Physiol (Lond)* 1997;498: 277–294.
22. Bostock H, Sears TA, Sherratt RM: The spatial distribution of excitability and membrane current in normal and demyelinated mammalian nerve fibres. *J Physiol (Lond)* 1983;341:41–58.
23. Bostock H, Sharief MK, Reid G, Murray NMF: Axonal ion channel dysfunction in amyotrophic lateral sclerosis. *Brain* 1995;118:217–225.
24. Burke D, Miller TA, Kiernan MC, Mogyoros I: Activity-dependent modulation of excitability. *Muscle Nerve* 1995;18: 675–676.
25. Carley LR, Raymond SA: Threshold measurement: applications to excitable membranes of nerve and muscle. *J Neurosci Methods* 1983;9:309–333.
26. Chaudhry V, Rowinsky EK, Sartorius SE, Donchower RC, Cornblath DR: Peripheral neuropathy from taxol and cisplatin combination chemotherapy: clinical and electrophysiological studies. *Ann Neurol* 1994;35:304–311.
27. Cikurel K, Murray NMF, Bostock H: The use of threshold electrotonus in motor neurone disease. *Electroencephalogr Clin Neurophysiol* 1997;103:168.
28. David G, Barrett JN, Barrett EF: Activation of internodal potassium conductance in rat myelinated axons. *J Physiol (Lond)* 1993;472:177–202.
29. Frankenhaeuser B, Huxley AF: The action potential in myelinated nerve fibres of *Xenopus laevis*, as computed on the basis of voltage clamp data. *J Physiol (Lond)* 1964;171:302–315.
30. Franz P, Weigl P, Grafe P, Baker M, Bostock H: Changes in excitability of human motor axons during ischaemia. *Pflügers Arch* 1988;411:R152.
31. Gasser HS: Changes in nerve potentials produced by rapidly repeated stimuli and their relation to the responsiveness of nerve to stimulation. *Am J Physiol* 1935;111:35–50.
32. Gilliatt RW, Meer J: The refractory period of transmission in patients with carpal tunnel syndrome. *Muscle Nerve* 1990;13: 445–450.
33. Gilliatt RW, Willison RG: The refractory and supernormal periods of human median nerve. *J Neurol Neurosurg Psychiatry* 1963;26:136–147.
34. Goldman L, Albus JS: Computation of impulse conduction in myelinated fibres: theoretical basis of the velocity-diameter relation. *Biophys J* 1968;8:596–607.
35. Grafe P, Quasthoff S, Grosskreutz J, Alzheimer C: Function of the hyperpolarization-activated inward rectification in non-myelinated peripheral rat and human axons. *J Neurophysiol* 1997;77:421–426.
36. Hanauske AR, Schilling T, Heinrich B, Kau R, Herzog M, Quasthoff S, Bochtler H, Diergarten K, Rastetter J: Clinical phase I study of Paclitaxel followed by Cisplatin in advanced

head and neck squamous cell carcinoma. *Semin Oncol* 1995; 22:35–39.
37. Horn S, Quasthoff S, Grafe P, Bostock H, Renner R, Schrank B: Abnormal axonal inward rectification in diabetic neuropathy. *Muscle Nerve* 1996;19:1268–1275.
38. Ingram DA, Davis GR, Swash M: The double collision technique: a new method for measurement of the motor nerve refractory period distribution in man. *Electroencephalogr Clin Neurophysiol* 1987;66:225–234.
39. Ingram SL, Williams JT: Modulation of the hyperpolarization-activated current (I_h) by cyclic nucleotides in guinea-pig primary afferent neurons. *J Physiol (Lond)* 1996;492:97–106.
40. Ito H, Kanazawa A, Ohno A, Tanaka T, Miwa T, Fukuda T, Ueki A, Notoya Y: Effect of prostaglandin E_1, E_2 and I_2 derivatives and methyl-B_{12} on peripheral neuropathy in diabetic rats: effects on sciatic nerve cyclic AMP, sorbitol and myoinositol content, in Ward J, Goto Y (eds): *Diabetic Neuropathy*. Chichester, U.K., Wiley, 1990, pp 517–523.
41. Kaji R: Pathophysiology and clinical variants of multifocal motor neuropathy, in Kimura J, Kaji R (eds): *Physiology of ALS and Related Diseases*. Amsterdam, Elsevier, 1997, pp 85–98.
42. Kapoor R, Smith KJ, Felts PA, Davies M: Internodal potassium currents can generate ectopic impulses in mammalian myelinated axons. *Brain Res* 1993;611:165–169.
43. Kiernan MC, Mogyoros I, Burke D: Differences in the recovery of excitability in sensory and motor axons of human median nerve. *Brain* 1996;119:1099–1105.
44. Kiernan MC, Mogyoros I, Burke D: Changes in excitability and impulse transmission following prolonged repetitive activity in normal subjects and patients with a focal nerve lesion. *Brain* 1996;119:2029–2037.
45. Kiernan MC, Mogyoros I, Hales JP, Gracies J-M, Burke D: Excitability changes in human cutaneous afferents induced by prolonged repetitive activity. *J Physiol (Lond)* 1997;500: 255–264.
46. Kiernan MC, Hales JP, Gracies J-M, Mogyoros I, Burke D: Paraesthesiae induced by prolonged high frequency stimulation of human cutaneous afferents. *J Physiol (Lond)* 1997;501: 461–471.
47. Kimura J: Refractory period measurement in the clinical domain, in Waxman SG, Ritchie JM (eds): *Demyelinating Disease: Basic and Clinical Electrophysiology*. New York, Raven Press, 1981, pp 239–265.
48. Kodama M, Kaji R, Kojima Y, Hirota N, Kohara N, Shibasaki H, Bostock H, Kimura J: Threshold electrotonus in patients with amyotrophic lateral sclerosis: further experience with Japanese subjects. *Electroencephalogr Clin Neurophysiol* 1995;97: S172.
49. Kojima Y, Kaji R, Hirota N, Kohara N, Kimura J, Murray NMF, Bostock H: Threshold electrotonus in monomelic amyotrophy with spinal hemiatrophy. *Electroencephalogr Clin Neurophysiol* 1995;97:S172.
50. Kugelberg E: Accommodation in human nerves and its significance for the symptoms in circulatory disturbances and tetany. *Acta Physiol Scand* 1994;8(suppl 24):1–105.
51. Layzer RB: The origin of muscle fasciculations and cramps. *Muscle Nerve* 1994;17:1243–1249.
52. Mackel R: Properties of cutaneous afferents in diabetic neuropathy. *Brain* 1989;112:1359–1376.
53. McDonald WI, Sears TA: The effects of experimental demyelination on conduction in the central nervous system. *Brain* 1970;93:583–598.
54. Miller TA, Kiernan MC, Mogyoros I, Burke D: Activity-dependent changes in impulse conduction in normal cutaneous axons. *Brain* 1995;118:1217–1224.
55. Miller TA, Kiernan MC, Mogyoros I, Burke D: Activity-dependent changes in impulse conduction in a focal nerve lesion. *Brain* 1996;119:429–437.
56. Mogyoros I, Kiernan MC, Burke D: Strength-duration properties of human peripheral nerve. *Brain* 1996;119:439–447.
57. Mogyoros I, Kiernan MC, Burke D: Strength-duration properties of cutaneous and motor axons in carpal tunnel syndrome. *Muscle Nerve* 1997;20:508–510.
58. Mogyoros I, Kiernan MC, Burke D, Bostock H: Excitability changes in human sensory and motor axons during hyperventilation and ischaemia. *Brain* 1997;120:317–325.
59. Noble D, Stein RB: The threshold conditions for initiation of action potentials by excitable cells. *J Physiol (Lond)* 1966;187: 129–162.
60. Panizza M, Nilsson J, Roth BJ, Basser PJ, Hallett M: Relevance of stimulus duration for activation of motor and sensory fibers: implications for study of H-reflexes and magnetic stimulation. *Electroencephalogr Clin Neurophysiol* 1992;85:22–29.
61. Panizza M, Nilsson J, Roth BJ, Rothwell J, Hallett M: The time constants of motor and sensory peripheral nerve fibers measured with the method of latent addition. *Electroencephalogr Clin Neurophysiol* 1994;93:147–154.
62. Pape HC: Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. *Annu Rev Physiol* 1996;58:299–327.
63. Poole EW: Ischaemic and post-ischaemic paraesthesiae in motor neurone disease. *J Neurol Neurosurg Psychiatry* 1957;20: 225–227.
64. Quasthoff S, Grosskreuts J, Kühn M, Schilling T, Hanauske A: Taxol-cisplatin neuropathy caused by membrane depolarisation: an in vivo and in vitro study. *J Neurol* 1995;242(suppl 2):145.
65. Quasthoff S, Horn S, Grosskreutz J, Grafe P: Effects of ischaemia on threshold electrotonus of peripheral nerve in diabetic patients. *J Neurol* 1995;242:S51.
66. Raymond SA: Effects of nerve impulses on threshold of frog sciatic nerve fibres. *J Physiol (Lond)* 1979;290:273–303.
67. Röper J, Schwarz JR: Heterogeneous distribution of fast and slow potassium channels in myelinated rat nerve fibres. *J Physiol (Lond)* 1989;416:93–110.
68. Ritchie JM: Physiology of axons, in Waxman SG, Stys PK, Kocsis JD (eds): *The Axon*. Oxford, Oxford University Press, 1995, pp 68–96.
69. Ruitjen MWMM, Salle HJA, Kingma R: Comparison of two techniques to measure the motor nerve refractory period distribution. *Electroencephalogr Clin Neurophysiol* 1994;93:299–305.
70. Schilling T, Heinrich B, Kau R, Herzog M, Quasthoff S, Diergarten K, Rastetter J, Hanausk A-R: Paclitaxel administered over 3 h followed by cisplatin in patients with advanced head and neck squamous cell carcinoma: a clinical phase I study. *Oncology* 1997;54:89–95.
71. Schneider U, Jund R, Nees S, Grafe P: Differences in sensitivity to hyperglycemic hypoxia of isolated rat sensory and motor nerve fibers. *Ann Neurol* 1992;31:605–610.
72. Scholz A, Reid G, Vogel W, Bostock H: Ion channels in human axons. *J Neurophysiol* 1993;70:1274–1279.
73. Schwarz J, Eikhof G: Na currents and action potentials in rat myelinated nerve fibres at 20 and 37°C. *Pflügers Arch* 1987; 409:569–577.
74. Schwarz J, Reid G, Bostock H: Action potentials and membrane currents in the human node of Ranvier. *Pflügers Arch* 1995;430:283–292.
75. Shahani B, Davies-Jones GAB, Russell WR: Motor neurone disease: further evidence for an abnormality of nerve metabolism. *J Neurol Neurosurg Psychiatry* 1971;34:185–191.
76. Shefner JM, Dawson DM: The use of sensory nerve action potentials in the diagnosis of peripheral nerve disease. *Arch Neurol* 1990;47:341–348.
77. Stephanova DI, Bostock H: A distributed-parameter model of the myelinated human motor fibre: temporal and spatial distributions of action potentials and ionic currents. *Biol Cybern* 1995;73:275–280.
78. Stephanova DI, Bostock H: A distributed-parameter model of the myelinated human motor nerve fibre: temporal and spatial distributions of electrotonic potentials and ionic currents. *Biol Cybern* 1996;74:543–547.

79. Strupp M, Bostock H, Weigl P, Piwernetz K, Renner R, Grafe P: Is resistance to ischaemia of motor axons in diabetic subjects due to membrane depolarization? *J Neurol Sci* 1990;99: 271–280.
80. Strupp M, Jund R, Schneider U, Grafe P: Glucose availability and sensitivity to anoxia of isolated rat peroneal nerve. *Am J Physiol* 1991;261:E389–E394.
81. Stys PK, Ashby P: An automated technique for measuring the recovery cycle of human nerves. *Muscle Nerve* 1990;13: 750–758.
82. Stys PK, Waxman SG: Activity-dependent modulation of excitability: implications for axonal physiology and pathophysiology. *Muscle Nerve* 1994;17:969–974.
83. Stys PK, Waxman SG: Activity-dependent modulation of excitability (a reply). *Muscle Nerve* 1995;18:676–677.
84. Tasaki I: Nature of the local excitatory state in the nerve fiber. *Jpn J Physiol* 1950;126:367–379.
85. Taylor JL, Burke D, Heywood J: Physiological evidence for a slow K^+ conductance in human cutaneous afferents. *J Physiol (Lond)* 1992;453:575–589.
86. Thomas PK, Tomlinson DR: Diabetic and hypoglycemic neuropathy, in Dyck PJ, Thomas PK, Griffin JW, Low PA, Poduslo JF (eds): *Peripheral Neuropathy*. Philadelphia, Saunders, 1993, pp 1219–1250.
87. Vogel W, Schwarz JR: Voltage-clamp studies on axons: macroscopic and single-channel currents, in Waxman SG, Stys PK, Kocsis JD (eds): *The Axon*. Oxford, Oxford University Press, 1995, pp 257–280.
88. Weigl P, Bostock H, Franz P, Martius P, Muller W, Grafe P: Threshold tracking provides a rapid indication of ischaemic resistance in motor axons of diabetic subjects. *Electroencephalogr Clin Neurophysiol* 1989;73:369–371.
89. Weiss G: Sur la possibilite de rendre comparables entre eux les appareils servant a l'excitation electrique. *Arch Ital Biol* 1901;35:413–446.