Excitability properties of motor axons in patients with spontaneous motor unit activity

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Abstract

Objectives—Measures of nerve excitability provide information about biophysical properties of peripheral axons in disease states. One measure, the strength duration time constant (τSD), was previously reported to be prolonged in motor axons of patients with acquired neuromyotonia. The present study used a new protocol that applies a more comprehensive and sensitive panel of measures of axonal excitability, to determine firstly whether changes in τSD were present in a group of patients with evidence of spontaneous motor unit activity; and secondly, if such changes in τSD were present, whether other parameters of axonal excitability were affected, to clarify the mechanism of the change in τSD.

Methods—Eleven patients with both symptoms and EMG evidence of spontaneous motor unit activity were studied. Eight patients had autoimmune associated acquired neuromyotonia (aNMT) and three had the cramp fasciculation syndrome. The protocol first measured stimulus-response behaviour using two stimulus durations (from which the distribution of strength-duration time constants was estimated), and then threshold tracking was used to determine threshold electrotonus to 100 ms polarising currents, a current-threshold relation (indicating inward and outward rectification), and the recovery of excitability after supramaximal activation.

Results—The results were compared with previously published normal data. The value for τSD of motor axons in the patient group was 0.43 (0.02) ms (mean (SEM)), identical with the control value. Most other indices of axonal excitability, including those dependent on fast potassium channels, were also found to be normal. When compared with age matched controls however, the patients with acquired neuromyotonia had significantly greater late subexcitability after an impulse, greater excitability overshoots after depolarisation or hyperpolarisation, and more accommodation.

Conclusions—No clear evidence for the mechanism of ectopic discharge in these patients was obtained, probably because the activity was generated focally, and most often at the motor nerve terminals. The unexpected finding of increased excitability overshoots and accommodation compared with age matched controls, suggests a relative up regulation of slow potassium conductance, possibly as a consequence of the continuous motor unit activity.

Keywords: excitability; spontaneous activity; fasciculation; cramp

Fasciculation and cramp are common manifestations of neurological disease and may be caused by peripheral nerve hyperexcitability. In a recent study on patients with acquired neuromyotonia (aNMT), a condition attributed to nerve hyperexcitability,1 2 it was reported that the strength-duration time constant (τSD) of motor axons was prolonged.3 Some of these patients expressed antibodies to neuronal voltage-gated potassium channels (VGKC), thought to be involved in the pathogenesis of this disease,7 and it was hypothesised that the increase in τSD was also caused by a functional blockade of VGKC by antibodies.

The mechanism whereby anti-VGKC antibodies might increase τSD is not clear.1 Strength-duration time constant is a membrane time constant inferred from the relation between threshold current and stimulus duration. It has been defined as the ratio between the minimum charge threshold and the rheobase.1 3 Studies of τSD have shown that it is a nodal property, depending partly on the passive membrane time constant (the product of nodal capacitance and resting resistance) and partly on voltage-dependent membrane properties, particularly a persistent Na+ conductance active at subthreshold potentials.9

Membrane depolarisation increases τSD as although the passive time constant is reduced by an increase in K+ conductance, this effect is outweighed by the activation of Na+ channels.8 Thus blocking nodal K+ channels active at the resting potential could increase τSD directly, by increasing nodal resistance, whereas blocking either nodal or internodal K+ channels would cause membrane depolarisation and increase τSD by activating Na+ channels.

However, the anti-VGKC antibodies in aNMT are thought to be directed against fast K+ channels,9 which contribute little to nodal conductance.10 These channels are found in their greatest number in the paranodal and juxtaparanodal regions,11 where they can contribute to the resting potential, but where access of antibodies is restricted. Further information about the mechanism of the increase in τSD should be obtainable from other excitability measurements sensitive to mem-
branes, potential, and/or $K^+$, or both conductances, such as the recovery cycle after an impulse and threshold electrotoneus.10 11 Tomimatsu et al have reported finding anti-VGKCs antibodies capable of suppressing $K^+$ currents not only in patients with aNMT, but also in patients with myokymia of unknown origin.12 The question therefore arose as to whether patients with such clinical syndromes associated with nerve hyperexcitability also demonstrated changes in axonal membrane properties. The current study was undertaken to determine whether changes in $\tau_{SD}$ were present in a group of patients with evidence of spontaneous motor unit activity, and if so, to determine how other parameters of axonal excitability were affected, to clarify the mechanism of the change in $\tau_{SD}$. In addition, the study was the first to assess the utility in the clinical setting of a newly described method designed to measure multiple measures of axonal excitability.13

**Patients and methods**

Studies were performed on 11 patients (eight men, three women, aged 37–67 years). All patients gave informed consent and the study was approved by the South Sefton research ethics committee (Walton Centre). No patients were taking immunosuppressive drugs. Patients stopped taking medication for symptomatic control of their cramp and muscle twitching 1 to 7 days before testing (carbamazepine five patients, sodium valproate two patients, phenytoin one patient, lamotrigine one patient, no therapy two patients).

Patients were recruited from a specialised neuromuscular outpatient clinic having presented with symptoms suggestive of continuous motor unit activity including muscle cramps, twitching, and stiffness, affecting both proximal and distal muscle groups in more than one limb. In each patient there was EMG evidence of nerve hyperexcitability in at least one affected muscle. Most (eight patients) demonstrated the characteristic EMG discharges present in aNMT—namely, doublet, triplet, or multiplet single motor unit discharges having a high intraburst frequency of between 40–400/s.2 14 An example is illustrated in fig 1 (lower plot). The remaining three patients had generalised fasciculation on EMG and the clinical diagnosis was the cramp fasciculation syndrome (CFS).15 Nerve conduction studies were within normal limits for each of the patients. No patient had a family history of muscle overactivity.

All eight patients with aNMT had evidence of autoimmunity dysfunction. Serum samples from all 11 patients were tested using a $^{125}$I-a-dendrotoxin radioimmunoassay for anti-VGKCs antibodies, which has a sensitivity of about 50%.2 Three patients with aNMT were positive: one at high titre (382 pmol/l) and two at low positive titres (100–200 pmol/l). Eight patients were negative (titre<100 pmol/l). The five patients with aNMT seronegative for anti-VGKCs antibodies all had other findings suggestive of an immune disorder. Two patients had rheumatoid disease with high titre rheumatoid factor. One patient’s aNMT improved both clinically and on serial EMG recordings after plasma exchange. One patient had undergone thymectomy for a lymphocytic thymoma and had high titre anticholinesterase antibodies without clinical or EMG evidence of myasthenia gravis. One had paraneoplastic aNMT as she was found to have a small cell lung carcinoma 3 months after the date of the recordings.17 In the three patients with CFS, extensive serum autoimmuno screens were normal.

Studies were performed using a recently described protocol designed to measure a number of different nerve excitability parameters rapidly.14 Compound muscle action potentials were recorded from thenar muscles using surface electrodes over the abductor pollicis brevis, with the active electrode at the motor point and the reference on the proximal phalanx. The EMG signal was amplified (gain 1000, bandwidth 1.6 Hz to 2 kHz) and digitised by computer (486 PC) with A/D board (DT2812, Data Translation Inc, 100 Locke Drive, Marlboro, MA 01752–1192, USA.), using a sampling rate of 10 kHz. Stimulus waveforms generated by the computer were converted to current with a purpose built isolator constant current stimulator (maximum output ± 50 mA). The stimulus currents were applied via non-polarisable electrodes (Red Dot, 3M Health Care, D-46325 Borken, Germany), with the active electrode over the median nerve at the wrist, and the reference electrode about 10 cm proximal over muscle. Stimulation and recording were controlled by new software, written in BASIC (QTRAC version 4.3, copyright Institute of Neurology, London, UK with multiple excitability protocol TRONDHM).

Test current pulses of 0.2 ms or 1 ms were applied regularly at 0.5 s intervals, and combined with suprathreshold conditioning stimuli or subthreshold polarising currents as required. A complete recording sequence is illustrated in figure 1. The amplitude of the compound muscle action potential (CMAP) was measured from baseline to negative peak. For all tracking studies, the target CMAP was set to be 40% of the peak response. Skin temperature was monitored close to the stimulation site and was kept constant above 32°C.

Stimulus-response curves were recorded separately for test stimuli of durations 0.2 ms and 1 ms. The stimuli were increased in 6% steps, with two responses averaged for each step, until three averages were considered maximal (fig 1A). The stimulus-response data were used for several purposes. Firstly, the 1-ms peak response was used to set the target submaximal response (40% of peak) for threshold tracking for the remainder of the study. Secondly, the slope of the 1 ms stimulus-response curve was used in conjunction with the tracking error (deviation from the target) to optimise the subsequent threshold tracking. Finally, when the data were analysed, the ratio between the 0.2 ms and 1 ms stimuli required to evoke the same responses were used to estimate the strength-duration time.
constants and rheobases of axons of different threshold.

Prolonged subthreshold currents were used to alter the potential difference across the internodal axonal membrane, a process referred to as electrotonus. The changes in threshold associated with electrotonus normally have a similar time course to the changes in membrane potential and are known as threshold electrotonus. In the present protocol, test stimuli of 1 ms duration were used to produce the target CMAP (40% of maximal). Threshold tracking was used to record the changes in threshold induced by subthreshold polarising currents, 100 ms in duration, set to be +40% (depolarising) and −40% (hyperpolarising) of the control threshold current. The three stimulus combinations were tested in turn: test stimulus alone (to measure the control threshold current), test stimulus+depolarising conditioning current, and test stimulus+hyperpolarising conditioning current. Threshold was tested at 26 time points (maximum separation 10 ms) before, during, and after the 100 ms conditioning currents. Each stimulus combination was repeated until three valid threshold estimates were recorded, as judged by the response being within 15% of the target response, or alternate responses being either side of the target (fig 1 B).

The current-threshold relation was tested with 1 ms pulses at the end of subthreshold polarising currents lasting 200 ms (fig 1 C). The polarising current was altered in a ramp fashion from +50% (depolarising) to −100%

![Figure 1](https://www.jnnp.com)
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(1.9) years, temperature 32.3 (0.2) °C to the full group of 95% confidence intervals for a normal subject, based on the 29 controls in the previous study.14

The stimulus-response curves in fig 2 A are normal in shape, because the normalised traces in fig 2 B all fall within the normal limits, as do the stimulus-response slopes in figure 3 B. There was therefore no evidence of axons being affected non-uniformly by the disease.21

The peak CMAP amplitudes were smaller on average, than the normal controls (figs 2 A, 3 B), but this difference was not significant when the comparison was made with age matched controls. One patient with CFS had an abnormally high threshold (fig 2 A) and rheobase (fig 3 A), and there was a tendency for the patients to have higher thresholds on average than the controls (fig 2 A). However, the difference from age matched controls was not significant at the 1% level, and was in the opposite direction from one which could account for the continuous motor unit activity.

The current-threshold relations for each patient, calculated using the normalised threshold changes at the end of 200 ms current pulses, are plotted in fig 2 C. For each level of conditioning current, from +50% to −100% of the control threshold, the threshold change was measured, and the percentage threshold reduction plotted, depolarisation to the right and hyperpolarisation to the left. The current-threshold relation reflects the rectifying properties of the axon (both nodal and internodal axolemma), and the slope of the curve can be used to provide an estimate of the threshold analogue of input conductance. The steepening of the curve towards the top right results from outward rectification, an accommodative response to the depolarising current, associated with the activation of fast and slow K⁺ channels, the steeper the plot the greater the accommodation (rectification). The less prominent steepening towards the bottom left of the figure represents accommodation to the hyperpolarising current due to inward rectification, and thereby represents activation of the hyperpolarisation-activated conductance, IₚH.22

Curves for each of the patients studied were within the normal range, as were the minimum and resting slopes of the current/threshold relationship (fig 3 C). This suggests that the rectifying properties of the nerves studied remained intact.14

Strength-duration time constant was calculated from the thresholds measured using test pulses of two durations, 0.2 and 1.0 ms.16 20 Calculations were performed for different axonal populations, using the normalised stimulus-response data as in figure 2 B. Nine populations were used, starting from axons contributing to

H.22

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the responses between 5%-15% of maximal, then 15%-25%, further increasing in 10% batches up to the maximum of 85%-95%. These nine τSDs were plotted against their corresponding CMAP responses in fig 2 D, and all patient τSD measurements fell well within the normal limits. This is also shown in fig 3 A, where τSDs are plotted against rheobase for responses 35%-45% of maximal. The value for τSD of motor axons in the patient group was 0.43 (0.02) ms (mean (SEM)). This value is the same as that reported for the control group (0.43 (0.02) ms; mean (SEM)) and very similar to that reported elsewhere for normal nerve (0.46 (0.13) ms, mean (SD)). Significantly, there was no evidence for the high τSD reported by Maddison et al in some of their patients with aNMT. As with the thresholds for a 50% maximum CMAP in fig 2 A, the rheobases in fig 3 A were higher than normal on average, and well above the normal range in one patient with CFS. A technical explanation, perhaps related to the distance of the stimulating electrode from the underlying motor fascicles, may have been responsible, as nerve conduction studies were normal in this patient, as in the others.

Figure 2  Excitability data for all subjects superimposed on the 95% confidence intervals for a member of the control population taken from a previous study (broken ellipses). (A) Absolute stimulus-response relations indicated by plotting half maximal CMAP amplitude v stimulus for half maximal response (log-log coordinates); (B) normalised stimulus-response relations; (C) current-threshold relation; (D) distribution of strength-duration time constants; (E) threshold electrotonus; (F) recovery cycle. Threshold tracking performed using a test pulse of 1 ms duration. In plot A, filled circles=patients with aNMT, open circles=patients with CFS.
The changes in excitability associated with threshold electrotonus are illustrated in fig 2 E. As in previous studies, the changes in threshold are plotted as threshold reductions, with responses to depolarising currents upwards, as is normal for electrotonus. The fast changes in threshold that occur at delays of 0 and 100 ms are due to the rapid (<1 ms) changes in potential occurring at the nodes of Ranvier at the onset and offset of the polarising currents. The slower excitability changes are caused by slower potential changes occurring passively on the internodal membrane, and by ion channels with slow kinetics, especially slow potassium channels at the nodes. The traces from the patients all fell within the normal limits, and conventional indices derived from the threshold electrotonus recordings, including the early depolarising response TEd (10–20 ms) which can reflect fast potassium channel activity, also fell within normal limits (fig 3 E).

To complete the protocol, the recovery cycle (the absolutely and relatively refractory periods, the supernormal period, and the late subnormal period) was recorded. In fig 2 F the threshold changes are plotted with a logarithmic time scale, to show more clearly the early events in the cycle. The traces from the patients mostly fell within the normal limits, but between 20 and 70 ms, the late subexcitability was greater than normal in one patient with aNMT and less than normal in a patient with CFS (fig 3 F). The remaining patients

![Figure 3](https://www.jnnp.com)
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with aNMT had subexcitabilities in the upper half of the normal range, and the mean subexcitability of the aNMT group (19.4 (1.5)%, mean (SEM), n=8) was abnormally high, whether compared with the full control group (14.7 (0.7)%, n=29, p=0.006) or with age and temperature matched controls (12.2 (1.1)%, n=8, p=0.002). Even when the outlying patient was omitted, the aNMT group had significantly higher subexcitability (18.0 (0.8)%, n=7) than the matched controls (p=0.001). By contrast, although there was a tendency for the superexcitabilities of the patients with aNMT (−20.9 (1.3)%, n=8) to be less than in the full control group (−25.3 (1.0)%, n=29, p=0.04), they were not significantly different from the matched controls (−23.0 (1.7)%, n=8, p=0.35).

To obtain further information about the membrane properties underlying this abnormality in subexcitability, we tested another 10 excitability parameters (in addition to the 12 in fig 3), derived from the data in fig 2, to see if they were abnormal in the patients with aNMT. Statistically the most significantly abnormal parameter tested was the subexcitability that followed a depolarising current—that is, the undershoot after depolarising threshold electrotonus. The value of this parameter in patients with aNMT (−21.5 (0.9)%, n=8) was significantly higher than in the matched control group (−15.8 (0.7)%, n=8, p=0.0001), although not significantly different from the full control group (−19.3 (0.7)%, n=29, p=0.13), as this parameter varies with age and temperature. Similarly, the threshold overshoot (increased excitability) after a hyperpolarising current was significantly greater in patients with aNMT (19.2 (1.4)%, n=8) than in age and temperature matched controls (14.0 (0.7)%, n=8, p=0.005), but not in comparison with the unmatched controls (16.5 (0.7)%, n=29, p=0.07). These abnormalities in the overshooting of electrotonic excitability after a displacement of membrane potential, whether by a spike or by applied current stimuli, are illustrated in fig 4. Apart from these three, the only other parameter of the 22 tested that differed significantly (p<0.01) between the patients with aNMT and matched controls was S2 accommodation, measured from the depolarising threshold electrotonus curve by subtracting the plateau level at 90–100 ms from the peak threshold reduction. This quantity, which depends in part, as do the excitability overshoots, on slow potassium conductance, averaged 29.9 (1.1)% in the patients with aNMT and 24.5 (0.7)% in the age matched controls (p=0.001). No such tendency to increased overshoots or accommodation was discernible in the three patients with CFS.

**Figure 4** Comparison of patients with aNMT (n=8) with pooled normal controls (controls entire, n=29), and a subset of age matched control subjects (controls matched, n=8) for selected excitability parameters: (A) Late subexcitability; (B) threshold electrotonus depolarising undershoot; and (C) threshold electrotonus hyperpolarising overshoot. Results are expressed as mean (SEM) and marked by an asterisk where significant (2 tailed t test; * p<0.01; ** p<0.001).

**Discussion**

The present study has established that a protocol recently developed to record multiple measures of axonal excitability can be used in the clinical setting, is tolerable to the patient, and can be employed in an efficient manner. Using this protocol, we have shown that patients with immune-associated aNMT, or CFS, who have active symptoms and signs of nerve hyperexcitability, and in some cases EMG findings of spontaneous motor unit activity during the recordings, had no clear evidence of axonal membrane hyperexcitability when compared with healthy control subjects.

It has been reported that τ_{SD}, a measure of axonal excitability, can be prolonged in motor axons of patients with amyotrophic lateral sclerosis (ALS). More recently a similar finding was obtained in some patients with aNMT. Such an increase in τ_{SD} may indicate membrane depolarisation. Alternatively it may occur by means of an increase in a persistent sodium conductance, or by paranodal demyelination.

In the study on patients with ALS, it was suggested that a greater representation of a persistent Na\(^+\) conductance may be responsible for both the prolongation of τ_{SD} and the development of fasciculation. In patients with aNMT, it was proposed that in addition to the above mechanisms, anti-VGKC antibodies may contribute to the prolongation of τ_{SD} recorded from motor axons by means of an indirect effect on axonal membrane properties. By contrast, the present study has found that in patients with similar clinical syndromes associated with spontaneous motor unit activity, no prolongation in the τ_{SD} of motor axons was evident.

Strength-duration time constant is a nodal property of the axonal membrane. Voltage gated K\(^+\) channels are not uniformly distributed along the axonal membrane. In aNMT the antibodies are thought to act against fast K\(^+\) channels, of which there are very few present in the nodal region. Their density is greatest in the paranodal region where, provided the myelin sheath is intact, they should be protected from circulating autoantibodies. Studies of the effects of 4-aminopyridine, a blocker of fast potassium channels, on the electrical properties of myelinated axons have shown that superexcitability is increased, as is the early depolarising electrotonus, and there is...
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By contrast with the fast K⁺ channels, there is a high density of slow K⁺ channels present at nodes of Ranvier, a site more accessible to immune attack. However, the excitability parameters that depend most on slow K⁺ channels—namely, the late subnormal phase in the recovery cycle, the accommodation that occurs with depolarisation during threshold electrotonus, and the undershoot in excitability that follows—were within the normal range and on average significantly greater in the patients with aNMT than in the age matched control group. These changes, which suggest a relative up regulation of slow K⁺ channels in these patients, could not account for the ectopic discharges. They might, however, indicate an adaptive response to the continuous motor unit activity. Recently differences in the same excitability parameters have been reported between median and peroneal axons, and the suggestion made that slow K⁺ conductance may depend on axonal discharge patterns or peak firing rates.11

WHERE DO THE ECTOPIC IMPULSES ORIGINATE?

These findings prompt questions about the nature and origin of the generator producing the ectopic motor activity that occurs in patients with aNMT and related syndromes. In aNMT, as in other immune mediated diseases, symptom severity and EMG findings can vary over time often in an unpredictable pattern. This may be one explanation why some previously studied patients have a prolonged τSD during a single, short period of recording while our and other similar patients do not. By implication, it is possible that the results using our protocol would be less uniform if testing were repeated serially in individual patients.

In the patients studied, no evidence of axonal hyperexcitability was detected, even when they had spontaneous activity in the abductor pollicis brevis during the testing, and regardless of their anti-VGKC antibody status or evidence of a systemic autoimmune diathesis. It seems unlikely that this lack of abnormality reflected an ongoing effect of the patient’s drug therapy, in most cases the anticonvulsant carbamazepine. All patients remained symptomatic, albeit to a lesser degree, even while taking medication. Furthermore, all patients ceased their medication from 1 to 7 days before testing, at which stage it was possible to record spontaneous activity during testing (fig 1). Finally, τSD has been found to be similar whether or not patients are taking carbamazepine. In the study by Maddison et al, some patients with aNMT had a prolonged τSD while others had values in the normal range. Moreover, there was no relation between the prolongation in τSD and the presence or absence of serum anti-VGKC antibodies, or the presence or absence of spontaneous motor unit activity during the recordings. These findings, taken together with our results, suggest that, as other immune mediated neurological diseases (for example, myasthenia gravis, Guillain-Barré syndrome, and multifocal motor neuropathy) acquired autoimmune mediated neuromyotonia may be a focal or multifocal disorder.

Previous studies have shown that there is variability in the site of origin for spontaneous activity between patients with aNMT. When the present data are combined with previous studies on patients with aNMT, electrophysiological heterogeneity becomes apparent, suggesting that such patients are likely to exhibit a focal or multifocal rather than generalised excitatory axonopathy. The ectopic activity present in our patients was not caused by a generalised disturbance of motor axon membrane excitability. More likely it was generated focally at a site distal to the recording electrode such as the motor nerve terminal or adjacent nodes. As the motor nerve terminal is relatively unprotected by the blood-nerve barrier, this site is particularly vulnerable to autoantibody attack (for example, the Lambert-Eaton syndrome). An alternative hypothesis is that the spontaneous activity arose proximally at the level of the anterior horn cell or nerve root. The finding that some patients with aNMT have oligoclonal bands in their CSF, and that band IgG binds to CNS neurons in an identical pattern to serum containing anti-VGKC antibodies, may support this view.13

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