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 ($t_{SD}$), 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 $t_{SD}$ were present in a group of patients with evidence of spontaneous motor unit activity; and secondly, if such changes in $t_{SD}$ were present, whether other parameters of axonal excitability were affected, to clarify the mechanism of the change in $t_{SD}$.

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 and three currents, a current-threshold relation (indicating inward and outward rectification), and more 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.

Results—The results were compared with previously published normal data. The value for $t_{SD}$ 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 more 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,\(^1\) it was reported that the strength-duration time constant ($t_{SD}$) of motor axons was prolonged.\(^1\) Some of these patients expressed antibodies to neuronal voltage-gated potassium channels (VGKC), thought to be involved in the pathogenesis of this disease\(^2\), and it was hypothesised that the increase in $t_{SD}$ was also caused by a functional blockade of VGKC by antibodies.

The mechanism whereby anti-VGKC antibodies might increase $t_{SD}$ is not clear.\(^4\) 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.\(^6,8\) Studies of $t_{SD}$ 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.\(^3,7\) Membrane depolarisation increases $t_{SD}$ as although the passive time constant is reduced by an increase in K+ conductance, this effect is outweighed by the activation of Na+ channels.\(^3\) Thus blocking nodal K+ channels active at the resting potential could increase $t_{SD}$ directly, by increasing nodal resistance, whereas blocking either nodal or internodal K+ channels would cause membrane depolarisation and increase $t_{SD}$ by activating Na+ channels.

However, the anti-VGKC antibodies in aNMT are thought to be directed against fast K+ channels,\(^1\) which contribute little to nodal conductance.\(^1\) 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 $t_{SD}$ should be obtainable from other excitability measurements sensitive to mem-
branle potential, and/or K+, or both conduct-
ances, such as the recovery cycle after an
impact and threshold electrotonus.12 13 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 τSD 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 mech-
anism of the change in τSD. 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.14

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. Pa-
ients stopped taking medication for sympto-
matic control of their cramp and muscle
twitching 1 to 7 days before testing (car-
bamazepine five patients, sodium valproate two
patients, phenytin one patient, lamotrigine
one patient, no therapy two patients).

Patients were recruited from a specialised
neuromuscular outpatient clinic having pre-
sented with symptoms suggestive of continu-
ous motor unit activity including muscle
bends, 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 dis-
charges present in aNMT—namely, doublet,
triplet, or multiplet single motor unit dis-
charges having a high intraburst frequency
of between 40–400/s.2 11 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).13 Nerve con-
duction 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 autoimmune dysfunction. Serum samples
from all 11 patients were tested using a
125I-dendrotoxin radioimmunoassay for anti-VGKC 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 (ti-
itre<100 pmol/l). The five patients with aNMT
seronegative for anti-VGKC antibodies all had
other findings suggestive of an immune disor-
der. 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 lym-
phophyctic thymoma and had high titre antiace-
tylcholine receptor 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 autoim-
immune screens were normal.

Studies were performed using a recently
described protocol designed to measure a
number of different nerve excitability param-
eters rapidly.14 Compound muscle action po-
tentials were recorded from thenar muscles
using surface electrodes over the abductor pol-
licis 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 compu-
ter were converted to current with a purpose
built bi-directional 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 record-
ing were controlled by new software, written in
BASIC (QTRAC version 4.3, copyright Insti-
tute of Neurology, London, UK with multiple
excitability protocol TRONDHM).

Test current pulses of 0.2 ms or 1 ms were
applied regularly at 1 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 tem-
perature was monitored close to the stimula-
tion 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 1 A). 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 conjunc-
tion 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 Example of data recorded from each patient: upper three plots show amplitudes of stimuli, CMAP responses and delay between conditioning and test stimuli for four segments of recording A-D. The lowest plot illustrates one EMG trace in 14, during the prestimulus period, to show spontaneous motor unit activity, with the sweep separation corresponding to an amplitude measure of 30 µV. (A) Stimulus-response behaviour, stimulus duration 0.2 ms, then 1.0 ms. (B) Threshold electrotonus recording with 100 ms conditioning stimuli, 40% and −40% of threshold. (C) Current/threshold recording with 200 ms conditioning stimuli varied from 50% to −100% of threshold. (D) Recovery cycle, threshold changes 200 to 2 ms after single, supramaximal stimulus.
Excitability properties of motor axons in patients with spontaneous motor unit activity

Excitability properties of motor axons in patients with spontaneous motor unit activity were studied. In the previous study (age 39.4°C, temperature (32.5°C (0.5°C), years, mean (SEM)), skin temperatures above 31°C, n=29), the patients with aNMT were significantly older (53.6 (2.6) years, n=8), and slightly warmer (33.8 (0.6)°C). To allow for these differences, a subset of age and temperature matched controls was constructed by selecting controls over 43 years old with skin temperatures above 31°C. This matched control group did not differ significantly in age (32.1 (1.7) years, n=8) or temperature (32.8 (0.2)°C) from the patients with aNMT. Excitability parameters were compared between the patient and both control groups (full and matched) using two tailed t tests. As multiple excitability parameters were compared, p values>0.01 were not considered significant.

Results

As with studies using the new protocol in healthy subjects, no patient found the stimulation sequence painful. The complete recording sequence took 8.5–11.8 minutes, mean 9.4 minutes. All patients had continuing symptoms and signs of generalised spontaneous muscle overactivity on the day of the recording. During the recording three patients had evidence of spontaneous activity in the abductor pollicis brevis muscle. In one subject doublet discharges were recorded throughout the study, illustrated in fig 1.

Primary data from all the patients are plotted in fig 2, and selected pairs of parameters are plotted as scatter plots in fig 3. Open circles differentiate the small CFS group in figs 2 A and 3, and the dotted lines in each figure indicate the 95% confidence intervals for a normal subject, based on the 29 controls in the previous study.

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, and may be estimated reliably from the thresholds for just two pulse durations. Values for the excitability parameters obtained in the current study were compared with normative data established in a previous study. However, some excitability properties change with age and/or temperature. Whereas the three patients with CFS were similar in age and/or temperature, the normal controls in the previous study were matched for just two pulse durations. Values for the excitability parameters obtained in the current study were compared with normative data established in a previous study.

The final part of the protocol recorded the recovery of excitability after a supramaximal conditioning stimulus (fig 1 D). These changes were recorded at 18 conditioning test intervals, decreasing from 200 to 2 ms in roughly geometric progression. Three stimulus combinations were tested in turn: (1) unconditioned test stimulus (of 1 ms duration) alone, and (3) conditioning-test stimuli. The response to (2) was subtracted on line from the response to (3) before the test CMAP was measured, so that the conditioning maximal CMAP did not contaminate the measured response when the conditioning test interval was short. Each stimulus combination was repeated until four valid threshold estimates were obtained.

A geometric progression. Three stimulus combinations were tested in turn: (1) unconditioned test stimulus (of 1 ms duration) alone, and (3) conditioning-test stimuli. The response to (2) was subtracted on line from the response to (3) before the test CMAP was measured, so that the conditioning maximal CMAP did not contaminate the measured response when the conditioning test interval was short. Each stimulus combination was repeated until four valid threshold estimates were obtained.

Rheobase current and Vth were calculated offline from the stimulus-response data. The strength-duration curve for peripheral nerve axons is hyperbolic, not exponential, and the best curve fit for strength-duration data comes from Weiss’ equation. This empirical law relates stimulus charge (threshold current multiplied by its duration) to stimulus duration and may be estimated reliably from the thresholds for just two pulse durations. Values for the excitability parameters obtained in the current study were compared with normative data established in a previous study. However, some excitability properties change with age and/or temperature. Whereas the three patients with CFS were similar in age and/or temperature, the normal controls in the previous study were matched for just two pulse durations. Values for the excitability parameters obtained in the current study were compared with normative data established in a previous study.

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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 \( \tau_{so} \) s were plotted against their corresponding CMAP responses in fig 2 D, and all patient \( \tau_{so} \) measurements fell well within the normal limits. This is also shown in fig 3 A, where \( \tau_{so} \) s are plotted against rheobase for responses 35%-45% of maximal. The value for \( \tau_{so} \) 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 \( \tau_{so} \) 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](image.png)

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](image-url)
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 after a displacement of membrane potential, whether by a spike or by applied current, that is, the undershoot after depolarising current—ability that followed a depolarising current—abnormal parameter tested was the subexcitability of the aNMT group (19.4 (1.5)%, mean (SEM), 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 τSD, 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 τSD 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 τSD 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 τSD 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 τSD 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

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).
a small membrane depolarisation. There was no evidence of any such changes among our patients.

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.

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 $\tau_{SD}$ 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, $\tau_{SD}$ 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 $\tau_{SD}$ while others had values in the normal range. Moreover, there was no relation between the prolongation in $\tau_{SD}$ 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 some 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. Theectopic activity present in our patients was not caused by a generalised disturbance of CNS neurone 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.

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