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Corticomotoneuronal function and hyperexcitability in acquired neuromyotonia

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Acquired neuromyotonia encompasses a group of inflammatory disorders characterized by symptoms reflecting peripheral nerve hyperexcitability, which may be clinically confused in the early stages with amyotrophic lateral sclerosis. Despite a clear peripheral nerve focus, it remains unclear whether the ectopic activity in acquired neuromyotonia receives a central contribution. To clarify whether cortical hyperexcitability contributes to development of clinical features of acquired neuromyotonia, the present study investigated whether threshold tracking transcranial magnetic stimulation could detect cortical hyperexcitability in acquired neuromyotonia, and whether this technique could differentiate acquired neuromyotonia from amyotrophic lateral sclerosis. Cortical excitability studies were undertaken in 18 patients with acquired neuromyotonia and 104 patients with amyotrophic lateral sclerosis, with results compared to 62 normal controls. Short-interval intracortical inhibition in patients with acquired neuromyotonia was significantly different when compared to patients with amyotrophic lateral sclerosis (averaged short interval intracortical inhibition acquired neuromyotonia $11.3 \pm 1.9\%$; amyotrophic lateral sclerosis $2.6 \pm 0.9\%$, $P < 0.001$). In addition, the motor evoked potential amplitudes (acquired neuromyotonia $21.0 \pm 3.1\%$; amyotrophic lateral sclerosis $38.1 \pm 2.2\%$, $P < 0.0001$), intracortical facilitation (acquired neuromyotonia $-0.9 \pm 1.3\%$; amyotrophic lateral sclerosis $-2.3 \pm 0.6\%$, $P < 0.0001$), resting motor thresholds (acquired neuromyotonia $62.2 \pm 1.6\%$; amyotrophic lateral sclerosis $57.2 \pm 0.9\%$, $P < 0.05$) and cortical silent period durations (acquired neuromyotonia 212.8 ± 6.9 ms; amyotrophic lateral sclerosis 181.1 ± 4.3 ms, $P < 0.0001$) were significantly different between patients with acquired neuromyotonia and amyotrophic lateral sclerosis. Threshold tracking transcranial magnetic stimulation established corticomotoneuronal integrity in acquired neuromyotonia, arguing against a contribution of central processes to the development of nerve hyperexcitability in acquired neuromyotonia.

Introduction

Acquired neuromyotonia refers to a group of disorders characterized by continuous ectopic nerve activity, manifesting clinically with cramps, fasciculations and stiffness (Hart *et al.*, 2002). These symptoms may be accompanied by hyperhidrosis, sensory abnormalities and central nervous system (CNS) features, referred to as Morvan's syndrome (Hart *et al.*, 2002). In ~40% of cases, antibodies against voltage-gated K$^+$ channels (VGKC) are present and may bind to both central and peripheral neurons (Hart *et al.*, 1997, 2002; Kleopa *et al.*, 2006).

Acquired neuromyotonia is characterized by the presence of spontaneous activity including positive sharp-waves and fibrillation potentials, fasciculations, myokymia, multiple discharges, muscle cramps and repetitive after-discharges in response to a voluntary contraction (Warmolts and Mendell, 1980; Hart *et al.*, 1997; Kiernan *et al.*, 2001). There have been varied findings regarding the origin of this ectopic activity in acquired neuromyotonia. Most studies have suggested that the ectopic focus arises at the unmyelinated distal nerve terminal (Isaacs, 1961; Wallis and Plum, 1969; Lutschg *et al.*, 1978; Lance *et al.*, 1979; Warmolts and Mendell, 1980; Auger, 1994; Deymeer *et al.*, 1998; Maddison *et al.*, 1999; Kiernan *et al.*, 2001; Hart *et al.*, 2002; Oh *et al.*, 2003). Antidromic propagation and axonal reflexes may also contribute to the generation of ectopic activity (Auger, 1994). Alternatively, it has been proposed that the ectopic focus may arise at either the anterior horn cell level, or even more centrally (Irani *et al.*, 1977; Hosokawa *et al.*, 1987; Hart *et al.*, 1996), as supported by the presence CNS symptoms and inflammatory changes, such as the presence of oligoclonal bands on cerebrospinal fluid analysis, in patients with acquired neuromyotonia (Newsom-Davis and Mills, 1993; Newsom-Davis, 1997; Liguori *et al.*, 2001; Hart *et al.*, 2002).

Separately, acquired neuromyotonia may be clinically misdiagnosed as amyotrophic lateral sclerosis (ALS) (Rowland and Shneider, 2001), particularly in the early stages of ALS where widespread fasciculations may be evident in the absence of other clinical features of ALS (Hirota *et al.*, 2000; Shiga *et al.*, 2000; de Carvalho and Swash, 2004; Kleine *et al.*, 2008). Of relevance, cortical hyperexcitability has been associated with the development of widespread fasciculations in ALS (Kaji *et al.*, 1993; de Carvalho *et al.*, 2000; Hirota *et al.*, 2000; Shiga *et al.*, 2000; Kleine *et al.*, 2008), although there have been limited, if any, studies that assessed the cortical function in acquired neuromyotonia.

Cortical function may be clinically assessed using paired-pulse transcranial magnetic stimulation (TMS) techniques (Vucic *et al.*, 2006), which had established that cortical hyperexcitability is an early feature in sporadic ALS (Vucic and Kiernan, 2006*b*), and precedes the clinical onset of familial ALS (Vucic *et al.*, 2008). Consequently, the present study used novel paired-pulse TMS techniques (Vucic *et al.*, 2006) to assess whether cortical hyperexcitability was evident in acquired neuromyotonia, and whether the presence of cortical hyperexcitability in ALS would enable one to differentiate the two diseases early in the disease process, before the development of the more characteristic neurophysiological changes of ALS.

Materials and methods

Patients

Studies were undertaken in 18 patients with acquired neuromyotonia (13 males, 5 females: age range 33–65 years, mean age: 50 years), diagnosed according to previously established criteria with symptoms or signs of muscle twitching or muscle cramps affecting at least two regions of skeletal muscles (Hart *et al.*, 2002). All patients demonstrated the characteristic EMG discharges consisting of doublet, triplet or multiplet single motor unit discharges with a high intraburst frequency of between 40–400/s (Fig. 1) (Hart *et al.*, 1997; Kiernan *et al.*, 2001). Patients were clinically assessed with muscle strength grades using the Medical Research Council clinical grading of power (Medical Research Council, 1976). Serum samples from all patients suspected of a diagnosis of acquired neuromyotonia were tested using a ^{125}I-α-dendrotoxin radioimmunoprecipitation assay for anti-VGKC antibodies, which has a sensitivity of approximately 50% (Hart *et al.*, 1997; Kiernan *et al.*, 2001).

Studies were also undertaken on 104 patients with definite or probable ALS (66 males, 38 females, aged 25–80 years, mean age: 58 years) as defined according to the revised El Escorial criteria (Brooks *et al.*, 2000). The mean disease duration to the time of testing was 18 ± 1.8 months, with a mean ALS functional rating scale-revised score of 39 ± 0.9. There was moderate weakness of the target
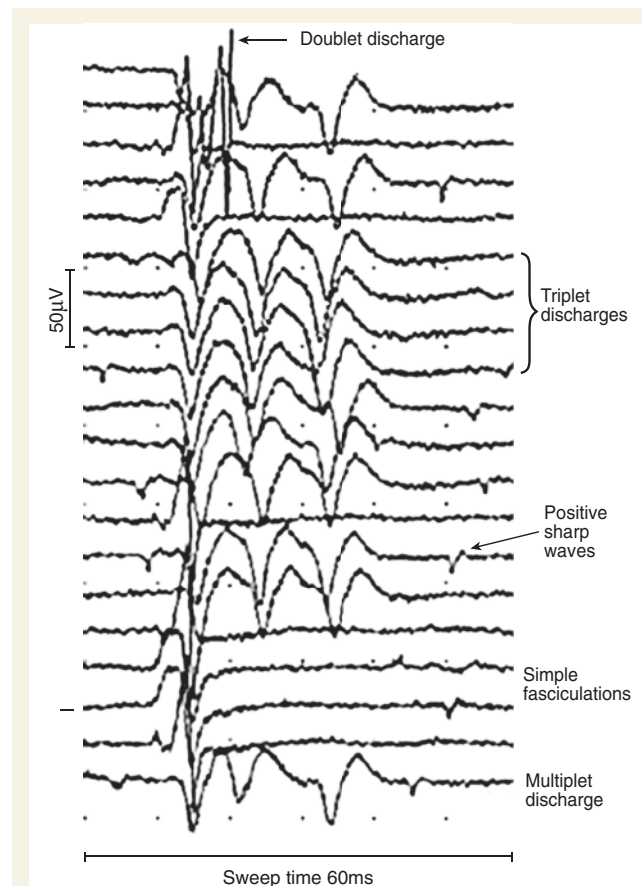
Figure 1 A raster display of doublet and triplet discharges along with fasciculations in a representative patient with acquired neuromyotonia (Patient 18, Table 1).

abductor pollicis brevis muscle, with a median Medical Research Council score of 4. Limb-onset disease was reported by 77% of patients with ALS, while bulbar-onset disease was evident in 23% of patients. Clinically, patients with ALS exhibited less fasciculations when compared to the acquired neuromyotonia cohort, although a formal fasciculation frequency was not calculated. Patients with ALS and acquired neuromyotonia cohort were clinically quantified using the same method. Using the compound muscle action potential (CMAP) amplitude as a broad marker of disease severity, with CMAP amplitude <4 mV a cut-off for more advanced disease (Vucic and Kiernan, 2006b), it was evident that the majority of patients with ALS (61%) exhibited less severe disease at the time of testing. Results from acquired neuromyotonia and patients with ALS were compared to control data obtained from 62 normal controls (31 males; 31 females, aged 23–83 years, mean: 46 years) (Vucic and Kiernan, 2006b). All patients gave informed consent to the procedures, which were approved by the South East Sydney Area Health Service Human Research Ethics Committee.

Peripheral nerve studies

Prior to undertaking cortical excitability studies, conventional nerve conduction studies were undertaken on all patients. The CMAP was recorded from the abductor pollicis brevis muscle as were the distal motor and F-wave latencies. From the CMAP amplitude, the neurophysiological index was calculated according to a previously established formula (de Carvalho and Swash, 2000).

Cortical excitability

Cortical excitability was assessed by applying a 90 mm circular coil to the motor cortex oriented to induce current flow in a posterior-anterior direction. The coil was adjusted until the optimal position for a motor evoked response was obtained from the abductor pollicis brevis muscle. Currents were generated by two high-power magnetic stimulators which were connected via a BiStim (Magstim Co., Whitland, South West Wales, UK) such that conditioning and test stimuli could be independently set and delivered through the one coil.

In the conventional paired-pulse technique, the conditioning and test stimuli are kept at constant intensity, and changes in the motor evoked potential (MEP) amplitude are measured. In the present study the amplitude of the MEP response was fixed and changes in the test stimulus intensity required to generate this target response, when preceded by either sub- or supra-threshold conditioning stimuli, were measured as previously described (Vucic et al., 2006).

The threshold tracking strategy used a target response of 0.2 mV as previously reported (Fisher et al., 2002; Vucic et al., 2006). Resting motor threshold was defined as the stimulus intensity required to produce and maintain this target MEP response. Initially, the stimulus response (SR) curve for cortical stimulation was determined by increasing the intensity of the magnetic stimulus to the following levels: 60, 80, 90, 100, 110, 120, 130, 140 and 150% resting motor threshold. Three stimuli were delivered at each level of stimulus intensity. Although the SR curve was recorded in all patients, the maximum MEP amplitude (mV) and MEP onset latency (ms), recorded at 150% resting motor threshold, was used in group comparisons. Central motor conduction time (cortical silent period, ms) was calculated according to the F-wave method formula (Mills and Murray, 1986):

$$CMCT = MEP \text{ latency} - [DML + F_{min} - 1]/2$$

where DML represents the distal motor latency and F_{min} the minimum F-wave latency.

Cortical silent period was induced by single-pulse TMS and recorded while patients performed a weak voluntary contraction. The duration of the silent period was measured from the beginning of MEP to the return of EMG activity (Cantello et al., 1992).

Short interval intracortical inhibition (SICI) and intracortical facilitation were measured according to previously devised threshold tracking protocols (Vucic et al., 2006). SICI was determined by using subthreshold conditioning stimuli (70% resting motor threshold) at increasing interstimulus intervals delivered in a sequential order as follows: 1, 1.5, 2, 2.5, 3, 3.5, 4, 5 and 7 ms. Intracortical facilitation was determined over the following interstimulus intervals: 10, 15, 20 and 30 ms, using a subthreshold conditioning stimulus intensity set to 70% resting motor threshold. All neurophysiological parameters, including SICI and intracortical facilitation, were recorded with the target muscle at rest. The target muscle was closely observed for the presence of voluntary activity as recorded by surface electrodes and also viewed on a monitor, and the patients and subjects were asked to relax the target muscle if the examiner noted the appearance of any voluntary activity. SICI was measured as the increase in the test stimulus intensity required to evoke the target MEP. SICI was calculated off-line as follows (Fisher et al., 2002; Vucic et al., 2006):

$$SICI = [\text{Conditioned test stimulus intensity} - RMT]/RMT \times 100$$

Facilitation was measured as the decrease in the conditioned test stimulus intensity required to evoke a target MEP.

Recordings of CMAP and MEP were amplified and filtered (3 Hz–3 kHz)using a GRASS ICP511 AC amplifier (Grass-Telefactor, Astro-Med Inc., West Warwick, RI, USA) and sampled at 10 kHz using a 12-bit data acquisition card (National Instruments PCI-MIO-16E-4). Data acquisition and stimulation delivery were controlled by QTRACS software (Institute of Neurology, Queen Square, London, UK).

Statistical analysis

Student's t-test and one-way analysis of variance were used to compare mean differences. A probability (P) value of < 0.05 was considered statistically significant. Results are expressed as mean ± standard error of the mean.

Results

The clinical features of the 18 patients with acquired neuromyotonia are summarized in Table 1. All patients exhibited typical clinical features of acquired neuromyotonia including generalized cramps, fasciculations and stiffness. On EMG testing, doublet, triplet, multiple motor unit discharges and fasciculations were reported in all patients, in keeping with previous reports (Newsom-Davis and Mills, 1993; Kiernan et al., 2001; Hart et al., 2002). Anti-VGKC antibodies were present in 33% of patients with acquired neuromyotonia. One patient exhibited a sensorimotor peripheral neuropathy with associated weakness of distal muscle groups in the upper and lower limbs, including weakness of thumb and finger abduction, ankle dorsiflexion and flexion and extension of the toes, all exhibiting an MRC grade 4.

Of relevance to the clinical overlap between acquired neuromyotonia and ALS, particularly in the early disease stages, 40% of patients with acquired neuromyotonia from the present series were initially erroneously diagnosed with ALS. Over the time of

Table 1 Clinical details for the 18 patients with acquired neuromyotonia

Patients	Age (years) sex	Fasciculations/ neuromyotonia	CNS features (mood disturbance, insomnia and confusion)	Anti-VGKC antibodies	Autoimmune accompaniments
1	40M	Present	Yes	Negative	Yes
2	74M	Present	Yes	Negative	Yes
3	71M	Present	No	Negative	No
4	58M	Present	Yes	Negative	Yes
5	17M	Present	Yes	Positive	No
6	35M	Present	Yes	Negative	Yes
7	43F	Present	No	Negative	No
8	59M	Present	Yes	Negative	Yes
9	58M	Present	No	Negative	Yes
10	61F	Present	No	Negative	No
11	38M	Present	Yes	Negative	Yes
12	52M	Present	No	Negative	No
13	51F	Present	No	Negative	No
14	23M	Present	No	Positive	Yes
15	47F	Present	Yes	Positive	Yes
16	53F	Present	No	Positive	No
17	56M	Present	No	Positive	No
18	55M	Present	Yes	Positive	No
Mean (SEM)	50 (5.6)				

Fasciculation potentials and neuromyotonia were evident in all patients with acquired neuromyotonia. Antibodies to VGKC were evident in 33% of patients. The phenotype expression of CNS was evident in 50% of patients with acquired neuromyotonia. Other autoimmune accompaniments, including myasthenia gravis, diabetes mellitus and rheumatoid arthritis were evident in 50% of patients.

clinical follow-up, the diagnosis of ALS was eventually excluded in all patients based on further clinical assessment, neurophysiological investigation and lack of clinical progression.

Peripheral nerve studies

The CMAP amplitude (acquired neuromyotonia 8.3 ± 1.1 mV; controls 10.4 ± 0.7 mV) and neurophysiological index (acquired neuromyotonia 1.8 ± 0.3; controls 2.5 ± 0.2) were similar in patients with acquired neuromyotonia and controls. However, there was a significant difference in the CMAP amplitude (5.6 ± 0.3 mV, $P<0.01$) and neurophysiological index (0.7 ± 0.1, $P<0.01$) between acquired neuromyotonia and patients with ALS, being reduced in the latter group.

Cortical excitability

The motor cortex was excitable in all patients with acquired neuromyotonia. Central motor conduction time was comparable across all groups (acquired neuromyotonia 5.3 ± 0.3 ms; ALS 5.3 ± 0.2 ms; controls 5.3 ± 0.2 ms). The resting motor threshold, defined as the unconditioned stimulus intensity required to produce and maintain the target MEP response, was significantly greater in acquired neuromyotonia (acquired neuromyotonia $62.2 \pm 1.6\%$; controls $60.0 \pm 1.0\%$), when compared to ALS (ALS $57.2 \pm 0.9\%$, $F=3.4$, $P<0.05$). Furthermore, the MEP amplitude, expressed as a percentage of the CMAP amplitude recorded following electrical stimulation, was significantly different between acquired neuromyotonia ($21.0 \pm 3.1\%$; controls $24.7 \pm 1.8\%$) and patients with ALS ($38.1 \pm 2.2\%$, $F=77.8$, $P<0.0001$).

There was no significant difference in the cortical silent period ($F=1.592$, $P=0.211$), MEP amplitude ($F=1.184$, $P=0.312$) and resting motor threshold ($F=1.483$, $P=0.235$) in acquired neuromyotonia patients with CNS features compared to acquired neuromyotonia patients without CNS features and controls. In addition, there was no significant difference in the cortical silent period ($F=1.450$, $P=0.241$), MEP amplitude ($F=2.195$, $P=0.119$) and resting motor threshold ($F=2.224$, $P=0.116$) in patients with acquired neuromyotonia that tested positive for anti-VGKC antibodies compared to those patients with acquired neuromyotonia that tested negative for anti-VGKC antibodies and controls.

SICI

SICI has been defined as an increase in the test stimulus intensity required to track a constant target MEP of 0.2 mV. In normal controls, two peaks of SICI were evident, a smaller peak at interstimulus interval of 1 ms and a larger peak at 3 ms. SICI was significantly different between acquired neuromyotonia and ALS, reduced in the latter, and consistent with cortical hyperexcitability (Fig. 2). In addition, peak SICI at an interstimulus interval of 1 ms (acquired neuromyotonia $8.3 \pm 1.5\%$; controls $7.5 \pm 0.8\%$; ALS, $1.7 \pm 0.7\%$, $F=16.1$, $P<0.001$; Fig. 3A) and at interstimulus interval 3 ms (acquired neuromyotonia $17.1 \pm 2.8\%$; controls $16.1 \pm 1.2\%$; ALS $5.4 \pm 1.2\%$, $F=15.4$ $P<0.001$; Fig. 3B) were significantly different. In further comparisons to acquired neuromyotonia, limb-onset ALS patients, who clinically exhibited more frequent fasciculations than bulbar-onset ALS patients, disclosed significantly reduced SICI ($4.3 \pm 1.4\%$, $P<0.01$).
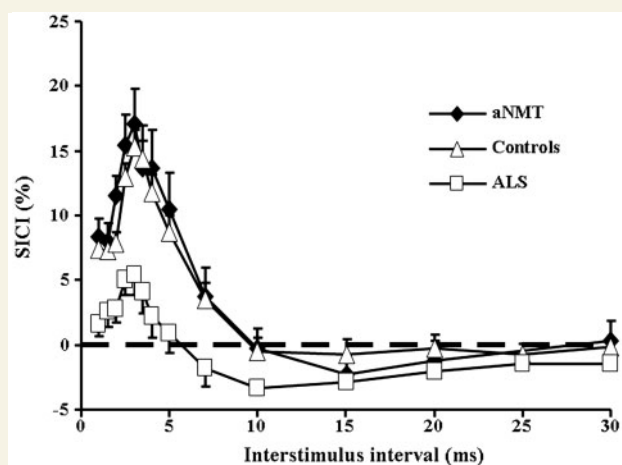
Figure 2 Short interval intracortical inhibition (SICI), defined as an increase in the test stimulus intensity required to track a constant target MEP response of 0.2 mV, was significantly different between acquired neuromyotonia (aNMT) and amyotrophic lateral sclerosis (ALS) patients.
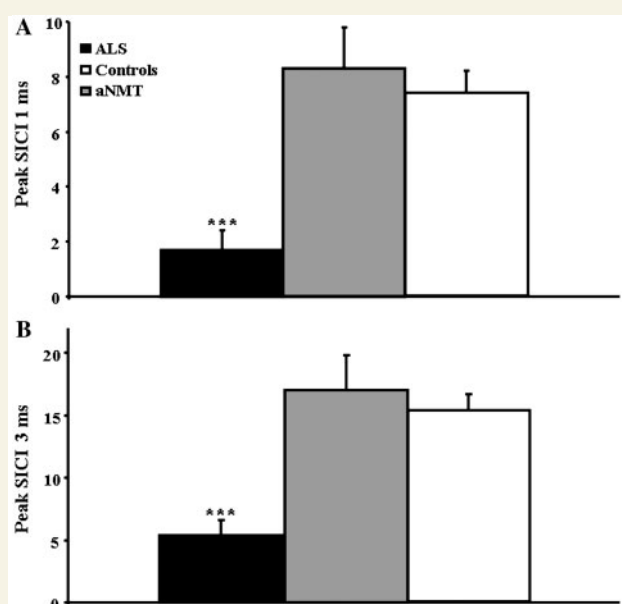
Figure 3 Peak short interval intracortical inhibition (SICI) at interstimulus interval of (**A**) 1 ms and (**B**) 3 ms was significantly different between acquired neuromyotonia (aNMT) and amyotrophic lateral sclerosis (ALS) patients, reduced in the latter and consistent with cortical hyperexcitability. ***$P < 0.001$.

Sub-group analysis revealed that averaged SICI was not significantly different in patients with acquired neuromyotonia with or without CNS symptoms, or when compared to controls ($F = 2.40$, $P = 0.10$). In addition, there was no significant difference of averaged SICI in patients with acquired neuromyotonia that tested positive for anti-VGKC antibodies, as compared to those that tested negative for anti-VGKC antibodies and controls ($F = 2.207$, $P = 0.118$).

Following SICI, a period of intracortical facilitation developed, marked by a decrease in the test stimulus intensity required to maintain the target MEP of 0.2 mV (Vucic *et al.*, 2006). Although there was no significant difference in peak intracortical facilitation at interstimulus interval 15 ms ($P = 0.37$), there was a significant difference of averaged intracortical facilitation, from interstimulus interval of 10–30 ms, between acquired neuromyotonia and patients with ALS, being increased in the latter (acquired neuromyotonia $-0.9 \pm 1.3\%$; controls $-0.8 \pm 0.7\%$; ALS $-2.3 \pm 0.6\%$, $F = 64.8$, $P < 0.0001$ Fig. 2).

Cortical silent period

In a contracting muscle, MEP is followed by a period of electrical silence that interferes with ongoing EMG activity, known as the cortical silent period. In the present series, the increase in the cortical silent period recruitment curve was non-linear in patients with acquired neuromyotonia, similar to the relationship established previously for control subjects (Vucic *et al.*, 2006). As stimulus intensity increased from 60 to 150% resting motor threshold, the mean cortical silent period duration increased from 0 to 212.8 ± 6.9 ms in patients with acquired neuromyotonia and was significantly different to patients with ALS, where it was reduced (ALS $0-181.1 \pm 4.3$ ms; controls 208.2 ± 3.1 ms, $F = 13.2$, $P < 0.0001$).

Discussion

Using novel threshold tracking TMS techniques the present study has established, for the first time, functional integrity of corticomotoneurons in patients with acquired neuromyotonia, arguing against a significant central contribution to the process of nerve hyperexcitability, and clearly differentiating acquired neuromyotonia from ALS. In addition, there was no difference in cortical excitability in acquired neuromyotonia patients with or without CNS symptoms, or those testing positive or negative for anti-VGKC antibodies. In contrast, the present study suggests that cortical hyperexcitability is specific to ALS across the spectrum of clinical presentations that report hyperexcitability and that, since the threshold tracking TMS technique reliably differentiates acquired neuromyotonia from ALS, it may be useful, particularly in the early disease stages where diagnostic confusion is possible.

What underlies the generation of ectopic activity in acquired neuromyotonia?

The presence of ectopic motor activity is a clinical hallmark in both acquired neuromyotonia and ALS (Rowland and Shneider, 2001; Hart *et al.*, 2002; de Carvalho *et al.*, 2008). In ALS, up-regulation of axonal persistent Na^+ conductances (Kanai *et al.*, 2006; Vucic and Kiernan, 2006*a*) and corticomotoneuronal mediated anterior horn cell hyperexcitability (Hirota *et al.*, 2000; Shiga *et al.*, 2000; Kleine *et al.*, 2008), have been identified as mechanisms underlying the generation of this ectopic activity. In acquired neuromyotonia, the mechanisms underlying the generation of this ectopic

activity are less clear. While some studies have reported that up-regulation of persistent Na$^+$ conductances underlies the generation of ectopic activity in acquired neuromyotonia, as indicated by an increase in the strength-duration time constant (Maddison *et al.*, 1999), this has not been a universal feature across acquired neuromyotonia cohorts (Kiernan *et al.*, 2001). The discrepancies in these peripheral findings strongly suggest that much of the ectopic activity in acquired neuromyotonia is generated at the motor nerve terminal (Isaacs, 1961; Deymeer *et al.*, 1998; Maddison *et al.*, 1999; Kiernan *et al.*, 2001; Hart *et al.*, 2002; Oh *et al.*, 2003).

Alternatively, it has also been suggested that ectopic activity in acquired neuromyotonia may arise at the level of the anterior horn cell in association with CNS dysfunction secondary to binding of anti-VGKC antibodies to central neurons (Irani *et al.*, 1977; Hosokawa *et al.*, 1987; Hart *et al.*, 1996; Newsom-Davis, 1997; Kleopa *et al.*, 2006). The finding of normal cortical excitability in patients with acquired neuromyotonia from the present series would argue against significant CNS dysfunction, and supports the notion that ectopic activity in acquired neuromyotonia predominantly arises peripherally. Specifically, blockade on the fast paranodal K$^+$ channels on the peripheral axon by anti-VGKC antibodies, may result in membrane depolarization, up-regulation of persistent Na$^+$ conductances and thereby generation of ectopic activity (Kiernan *et al.*, 2001). Recent evidence indicates that many of the high anti-VGKC antibodies are directed against other proteins that are intimately associated with anti-VGKCs such as Caspr2 at the juxtaparanodes, or Lgi1 in the molecular layer of the hippocampus (Irani SR *et al.*, submitted). The targets for the antibodies in patients with aNMT, however, have not yet been fully defined.

It remains a possibility that excessive activity in corticomotoneurons, leading to activity-dependent hyperpolarization through activation of the Na$^+$–K$^+$ pump (Vagg *et al.*, 1998; Vucic and Kiernan, 2007), could result in greater stimulus intensity or thresholds being required to generate the target MEP response and could have masked any underlying cortical hyperexcitability in acquired neuromyotonia. However, this seems an unlikely explanation for the findings since both SICI and intracortical facilitation were measured by subtracting the conditioning-stimulus intensity (Channel 3) from the unconditioned stimulus intensity (Channel 1, see 'Materials and methods' section), thereby subtracting out any 'noise' activity, or excessive stimulus intensity from the final calculations.

In accordance to changes in SICI, the MEP amplitude was increased in patients with ALS, most probably reflecting cortical hyperexcitability and in keeping with previous reports (Eisen *et al.*, 1993; Zanette *et al.*, 2002; Vucic and Kiernan, 2006*b*, 2008; Vucic *et al.*, 2008). Alternatively, it could be argued that the increase in the MEP amplitude reflects the presence of fewer and large motor units in ALS. This notion may be supported by previous studies using the triple stimulation technique that revealed a maximal TMS stimulus was capable of activating nearly all motor neurons supplying the target muscle (Magistris *et al.*, 1998). In contrast to the triple stimulation technique findings, single pulse TMS studies have established that the MEP amplitudes were normal in conditions with a comparable degree of lower motor neuron loss, such as Kennedy's disease (X-linked spinobulbar muscular atrophy) and

hereditary motor neuronopathy with pyramidal signs (Vucic and Kiernan, 2008; Vucic *et al.*, 2010). Furthermore, longitudinal peristimulus time histogram studies in patients with ALS did not report an increase in the primary peak amplitude, which might be expected with the presence of large motor units (Weber *et al.*, 2000). Although considered less likely, the possibility that larger and fewer motor units are contributing to the increased MEP amplitude in the present study cannot be discounted.

Diagnostic utility of threshold tracking TMS

The diagnosis of acquired neuromyotonia relies on the presence of clinical symptoms combined with neurophysiological findings of doublet, triplet or multiplet ('myokymic') motor unit discharges and fasciculations (Hart *et al.*, 2002). Although the clinical features of acquired neuromyotonia and ALS may be different, with patients with acquired neuromyotonia exhibiting a faster fasciculation frequency (Hart *et al.*, 2002), in certain clinical scenarios acquired neuromyotonia may be misdiagnosed as ALS. This may be the case when cramps and fasciculations are accompanied by clinical weakness, as may occur when acquired neuromyotonia is associated with a motor polyneuropathy (Newsom-Davis, 1997; Hart *et al.*, 2002). Moreover, anti-VGKC antibodies >100 pM were reported in up to 5% of individuals >50 years (Vincent *et al.*, 2004), and recently in 30% of patients with ALS (Nwosu *et al.*, 2010), although the latter study did not state the cut-off for normal values recommended by the laboratory involved.

In addition, fasciculations may be the sole presenting feature of ALS (Gubbay *et al.*, 1985), developing prior to fibrillation potentials, positive sharp waves and chronic neurogenic changes (de Carvalho *et al.*, 1999; de Carvalho and Swash, 2004). Such presentations may result in misdiagnosis and ultimately a delay in the institution of appropriate management. The contrasting finding of cortical excitability suggests that the threshold tracking TMS technique may be of diagnostic utility in differentiating acquired neuromyotonia from ALS, particularly in the early disease stages.

Funding

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