Clinical Neurophysiology 128 (2017) 1075–1082

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

The evolution of motor cortical dysfunction in amyotrophic lateral sclerosis

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See Editorial, pages 1032–1033

ARTICLE INFO

Article history:
Accepted 1 March 2017
Available online 15 March 2017

Keywords:
Amyotrophic lateral sclerosis
Transcranial magnetic stimulation
Inhibitory interneuron
Intracortical inhibition
Disease stage
Degeneration

HIGHLIGHTS

- Alterations of cortical function in amyotrophic lateral sclerosis have been investigated.
- Cross-sectional and longitudinal analyses revealed a gradual reduction in intracortical inhibition.
- These findings suggest dysfunction of inhibitory interneurons with disease progression.

ABSTRACT

Objective: The present study aimed to investigate alterations in cortical function in amyotrophic lateral sclerosis (ALS) related to disease progression.
Methods: In total, clinical assessments were evaluated in 189 ALS patients, combined with assessment of cortical function utilising threshold tracking transcranial magnetic stimulation. Results were compared with disease stage. Disease stage was defined in three ways: (1) as a proportion of disease duration in deceased patients; (2) from the time of ALS onset; and (3) using the ALS rating scale-revised (ALSFRS-R).
Results: Prospective studies in ALS patients demonstrated decreased neurophysiological index ($p < 0.0001$) and decreased compound muscle action potential (CMAP) ($p < 0.0001$), combined with abnormalities of central function including prolonged central motor conduction time (CMCT) ($p < 0.05$), increased motor evoked potential/CMAP amplitude ratio ($p < 0.0001$) and decreased short interval intracortical inhibition (SICI) ($p < 0.001$). SICI at 3 ms ($p < 0.05$, $\beta = -0.21$) and averaged SICI ($p < 0.05$, $\beta = -0.21$) decreased with disease progression, measured using proportion of disease duration. Alternatively, using time from disease onset, CMCT prolonged with disease progression ($p < 0.01$, $\beta = 0.25$), while ALSFRS-R decline correlated with decreased SICI at 3 ms ($p < 0.01$, $\beta = 0.20$).
Conclusions: Clinical measures combined with assessment of cortical function established that SICI decreased with disease progression.
Significance: These findings may suggest dysfunction of inhibitory interneurons with disease progression.

1. Introduction

The process of motor neuron death remains unresolved in amyotrophic lateral sclerosis (ALS) (Eisen et al., 1992; Vucic et al., 2013b), with a prevailing hypothesis that motor neuron hyperexcitability induces transsynaptic anterior horn cell degeneration via anterograde glutamate mediated excitotoxicity. Recent advances in techniques including transcranial magnetic stimulation (TMS), have provided support for this hypothesis. Specifically, cortical hyperexcitability has been determined to precede the onset of clinical weakness in carriers of genetic mutations linked to familial ALS; and may also develop as an early feature of spo-

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http://dx.doi.org/10.1016/j.clinph.2017.03.004

radic ALS (Vucic and Kiernan, 2006; Vucic et al., 2008). Even after the onset of clinical weakness, upper motor neuron hyperexcitability often precedes lower motor neuron dysfunction (Vucic and Kiernan, 2006; Menon et al., 2015b). Separately, riluzole produces glutaminergic modulation of cortical excitability, as determined by TMS techniques (Cheah et al., 2010; Vucic et al., 2013a). Taken in total, these findings suggest that cortical excitability is linked to the processes that underlie motor neuron death in ALS.

At a molecular level, multiple mechanisms may underlie and contribute to the development of motor neuron degeneration in ALS (Bae et al., 2013; Kuwabara et al., 2014; Simon et al., 2015). For instance, postmortem studies revealed that the excitatory amino acid transporter 2 becomes selectively impaired in the motor cortex and spinal cord of ALS patients, and this alteration may in turn inhibit reuptake of glutamic acid (Rothstein et al., 1995). Additionally, glutamate levels become increased in the CSF of ALS patients, a finding not observed across other neurodegenerative diseases (de Belleroche et al., 1984; Rothstein et al., 1990). Regardless of the cause, excessive glutaminergic activity may induce glutamate-induced excitotoxicity and result in neurodegeneration through activation of Ca^{2+}-dependent enzymatic pathways (Meldrum and Garthwaite, 1990). Of note, motor neurons in ALS patients express a defect in editing of the mRNA encoding the GluR2 subunit of glutamate AMPA receptors, resulting in increased Ca^{2+} permeability (Kawahara et al., 2004). Each of these changes seems likely to contribute to motor neuron degeneration in ALS.

Separately, alterations in intracortical inhibition have also been identified, with decreased intracortical inhibition further rendering motor cortical neurons hyperexcitable (Geevasinga et al., 2014; Geevasinga et al., 2015). Recently, the utility of cortical hyperexcitability as a signature of ALS, specifically decreased intracortical inhibition, has been considered as a diagnostic marker, with high sensitivity and specificity (Menon et al., 2015a). This functional information is supported by pathological studies that have demonstrated loss of parvalbumin-positive inhibitory interneurons in the motor cortex of ALS patients, with reductions in mRNA expression of the $GABA_A$ receptor subunit in the motor cortex and marked reduction of calbindin D-28 k immunoreactive cells in the frontal cortex, which are associated with GABA-ergic interneurons (Ferrer et al., 1993; Nihei et al., 1993; Petri et al., 2003).

While the clinical utility and importance of motor cortical function assessment has been established in ALS, changes in motor cortical function in relation to disease stage has not been defined. To date, limited studies have identified the relationship between ALS disease progression. These have consisted of TMS findings in relatively small patient cohorts, where there was limited consideration of phenotypic factors which may also influence cortical function, including age, gender, site of onset and administration of riluzole (Prout and Eisen, 1994; Ziemann et al., 1997; Smith et al., 1999; Zanette et al., 2002; Mills, 2003; Floyd et al., 2009; McGinley et al., 2010; Vucic et al., 2011, 2013a). As such, the present study was undertaken in a large cohort of ALS patients, with cortical function charted against disease onset and stage of the disease, clinical features, central and peripheral functional studies, to elucidate the changes in cortical function that occur and develop throughout the course of disease.

2. Methods

2.1. Subjects

Patients were recruited from multidisciplinary ALS clinics as part of the NHMRC Sydney Health Partners Program and were included in the analyses if they fulfilled the revised El Escorial criteria for definite or probable ALS (Brooks et al., 2000; Kanai et al., 2012). Additionally, patients initially diagnosed as possible ALS, who were observed until death and subsequently confirmed as ALS were also included. Patients with a family history of motor neuron disease, genetically proven familial ALS and those in whom a motor evoked potential (MEP) could not be obtained were excluded from this cohort. Patients with progressive muscular atrophy and primary lateral sclerosis were excluded from the study.

Clinical and neurophysiological data were prospectively collected. Clinical data collected included the date of symptom onset, gender, age at assessment, age at symptom onset, site of onset and administration of riluzole at the time of assessment. Longitudinal clinical follow-up was combined from the time of initial recruitment. Cortical and peripheral functional studies were compared with an age-matched cohort of 58 healthy subjects, who were not prescribed medications with known neurological effects. All subjects provided written informed consent, and the study was approved by the Human Research Ethics Committees of the South Eastern Sydney Local Health District and the Western Sydney Local Health District.

2.2. Disease staging

Disease stage was defined through the following approaches: (1) in deceased patients, the duration of disease at the time of cortical functional assessment from onset to death was normalized between zero (onset) and one (death) and expressed as a percentage (Mills, 2003); (2) in current, living ALS patients, the time in months from the onset of symptoms to the cortical function study and (3) Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) at the time of TMS testing were all utilised (Cedarbaum et al., 1999).

2.3. Cortical function

Threshold tracking TMS studies were undertaken using two high-power magnetic stimulators connected via a BiStim 200^2 system (Magstim Co., Whitland, South West Wales, UK) and a 90 mm circular coil was placed over the motor cortex and adjusted to evoke a MEP at the lowest possible stimulus intensity. Paired-pulse TMS was undertaken using the threshold tracking method as previously reported (Vucic et al., 2006), with changes in the test stimulus intensity required to generate a target MEP of 0.2 mV ± 20% measured recording over the abductor pollicis brevis (APB). Resting motor threshold was defined as the stimulus intensity required to generate this 0.2 mV ± 20% response.

Short interval intracortical inhibition (SICI) was measured by using subthreshold conditioning stimuli (70% resting motor threshold) at an interstimulus interval (ISIs) of 1 ms, 3 ms or averaged between 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, and 7 ms. Intracortical facilitation (ICF) was measured at ISIs of 10, 15, 20, 25 and 30 ms. Stimuli were delivered continuously over three channels: (1) tracking the stimulus intensity to maintain target MEP amplitude (i.e., resting motor threshold); (2) tracking the response to the subthreshold conditioning stimulus; and (3) tracking the stimulus required to maintain the target MEP amplitude following a sub threshold conditioning stimulus. SICI and ICF were measured as the change from the test stimulus intensity required to induce the target MEP, as per the following equation:

$$\text{Inhibition or Facilitation} = \frac{\text{Conditioned test stimulus intensity} - \text{Resting motor threshold}}{\text{Resting motor threshold}} \times 100$$

The maximum MEP amplitude (mV), MEP onset latency (ms) and cortical silent period (CSP) duration were measured using three stimuli of 150% RMT. Central motor conduction time (CMCT, ms) was calculated using the MEP onset latency and F-wave