Different patterns of I-waves summation in ALS patients according to the central conduction time


Department of Neuroscience, Psychiatric and Anaesthesiological Sciences University of Messina, Italy

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Abstract

Objectives: To study facilitatory I-waves interaction, using two near threshold stimuli, to test both excitability and conductivity changes related to cortico-motoneuronal involvement in amyotrophic lateral sclerosis (ALS) patients in different stages of the disease.

Methods: Pairs of threshold magnetic stimuli were applied over the motor cortex at inter-stimulus intervals (ISI) ranging from 1–1.5 to 2.5–3 ms and from 4 to 4.5 ms. The electromyogram responses were recorded from relaxed first dorsal interosseus (FDI).

Results: The data of I-waves summation were distributed according to the central conduction time (CCT) and all 3 peaks of facilitation were considered for statistical analysis. Patients with normal CCT showed a normal I-waves summation for the first peak, whilst patients with abnormal CCT had a significant reduction in facilitation \( P < 0.02 \). Six out of 11 patients with normal CCT had facilitation in the first peak, which exceeded 2 SD of normal values.

Conclusions: In conclusion ALS patients showed two different and opposite patterns of I-waves summation which could be related to different stages of the disease. © 2002 Published by Elsevier Science Ireland Ltd.

Keywords: Amyotrophic lateral sclerosis; I-waves summation; Transcranial magnetic stimulation

1. Introduction

Transcranial electrical or magnetic stimulation (TMS) is used to investigate the involvement of upper motor neuron (UMN) in patients with motor neuron diseases (Eisen et al., 1992; Ingram and Swash, 1987; Thompson et al., 1987). The TMS findings in amyotrophic lateral sclerosis (ALS) patients may vary significantly with respect to the clinical stage. At the beginning of the illness, motor evoked potentials (MEPs) with a low threshold and large amplitude can be recorded (Eisen et al., 1993; Desiato and Caramia, 1997). As the disease progresses threshold rises, and MEPs amplitude decreases. Transcranial magnetic stimulation of the human motor cortex using an intensity slightly above motor threshold elicits I-waves (indirect) in the cortico-spinal tract (Boyd et al., 1986). I-waves refer to high-frequency (approximately 600 Hz) repetitive discharge of cortico-spinal fibres produced by single-pulse stimulation of the motor cortex (Ziemann and Rothwell, 2000). First detected in animal preparations, this multiple discharge can also be recorded in humans with epidural electrodes over the spinal cord (Di Lazzaro et al., 1998). The exact nature of the generation of I-waves is still unclear, but there is convincing evidence that they originate in the motor cortex, mainly through activation of cortico-cortical projections onto cortico-spinal neurons. The physiology of I-waves can be non-invasively investigated through recently developed paired-pulse transcranial magnetic stimulation protocols (Tokimura et al., 1996; Ziemann et al., 1998a,b). The studies, so far, indicated that at very short intervals (1.2–1.5 ms) a facilitatory I-waves interaction occurs at the level of the motor cortex and that the mechanism responsible for the production of I-waves is under the control of gamma-aminobutyric acid (GABA) related inhibition (Ziemann et al., 1998b). Post-mortem and in vivo studies have shown that there is a loss of the glial glutamate transporter and an increase of glutamate concentrations in the motor cortex, spinal cord and CSF of ALS patients (Rothstein, 1995; Aoki et al., 1998). Moreover, several immunohistochemical studies have shown a selective loss of parvoalbumin immunoreactive GABAergic inter-neurons in ALS motor cortex (Nihei et al., 1993). These changes suggest that a loss of local circuit inhibitory control (Eisen et al., 1990; Enterzari-Taher et al., 1997; Nakajima et al., 1997) along with glutamate induced hyperexcitability,
within the motor cortex, may play a pivotal role in the degeneration of the cortico-motoneuronal system especially in the early stages of the disease. Short latency facilitation between pairs of threshold magnetic stimuli on the motor cortex of ALS patients has been recently assessed at inter-stimulus intervals (ISIs) 1–5 ms (Salerno and Georgesco, 2001). Compared to controls the facilitatory effects normally recorded at ISI 1 and 3 ms were considerably reduced suggesting an involvement in the circuits responsible for the generation of I-waves. However, there was no correlation between the I-waves summation and the disease duration. The aim of the present article was to correlate the I-waves summation with the central conduction time (CCT) which is the well-accepted marker of the integrity of cortico-spinal tract.

2. Methods

Thirty consecutive patients with clinically definite sporadic ALS, according to the revised ‘El Escorial Criteria’, were included in the present study (Brooks et al., 2000). Five patients were excluded because no compound motor action potential, after ulnar nerve stimulation, were elicitable or smaller than 3 mV. None of the included patients (mean age 56.3 ± 12 years; range 35–74 years; 14 men, 11 women) had any other obvious neurological disease. In all patients the diagnosis of ALS was confirmed by electromyogram (EMG) evidence of lower motor neuron degeneration in at least 3 (bulbar, cervical, thoracic, lumbosacral) regions. Three patients showed a pure bulbar form. The average disease duration was 7.1 months for the CCT normal group and 13.7 months in patients with abnormal CCT. For better assessment of disease status of the ALS, functional rating scale was used. (Cedarbaum and Stambler, 1997). The patients were all treated with riluzole. For comparison, 10 healthy age matched control subjects (mean age 56.8 ± 10.2 years; range 35–75 years; 5 men, 5 women) were investigated.

2.1. Procedures

All subjects were seated in a comfortable reclining chair. Surface EMG was recorded from the first dorsal interosseus (FDI) using 0.9 cm diameter silver/chloride surface electrodes with a belly-tendon montage. Responses were amplified using one channel with gain of 2000, and filtered by Neurolog System supplied by Digitimer with a time constant of 3 ms, and a high pass filter set at 3 kHz. Transcranial magnetic stimulation was performed by using two Magstim 200 HP magnetic stimulators (Magstim, Whitland, UK), which were connected through a bistim module to a figure of 8 shaped coil. The coil was placed flat on the skull with the handle pointing backwards and rotated about 45° away from the midline. Thus, the current induced in the brain was approximately perpendicular to the central sulcus. This orientation of the induced electrical field is thought to be optimal for a predominantly transsynaptic mode of activation of the cortico-spinal system. The position and site of the coil, for optimal activation of the target muscle, was determined where the stimulation produced consistently the largest MEPs at a slightly supra-threshold stimulus intensity. The threshold was determined according to the recommendation of the IFCN Committee (Rossini et al., 1994), and was defined as the intensity of stimulation which did not elicit at least 5 MEPs of 50 μV in 10 consecutive trials. I-waves summation was studied in a paired TMS protocol. The intensity of both stimuli, S1 and S2, was fixed at rest. ISIs from 0.5 to 4.5 ms, were tested in 0.2 ms steps (=20 ISIs). Each session consisted of two blocks of 120 trials each. Each block was composed of 12 conditions presented 10 times each: S1 and S2 given alone and 10 paired conditions (S1 + S2) at different ISIs.

The order of conditions within the blocks was randomised. The mean peak-to-peak size of each condition was measured and then expressed as a percentage of the computed sum of S1 + S2 given alone at that particular interval. Moreover, we determined the cut-off levels (±2 SD) of abnormality for the first peak of facilitation at 1.5 ms. Signals were collected via a CED 1401 laboratory interface (Cambridge Electronic Design, Cambridge, UK) and fed into a personal computer for offline analysis (sampling rate of 5 kHz per channel). Silent period duration (ms), obtained using a stimulus intensity of 130% of resting motor threshold, was measured from the end of the MEP (considered as the coming back of the motor response to the baseline) to the rebound of voluntary EMG activity.

Motor central conduction time (CCT-ms), was calculated by means of F-wave formula (Rossini et al., 1994). Informed consent was obtained from patients and controls according to the declaration of Helsinki and the protocol was approved by the Ethical Committee of our Institution.

2.2. Statistical analysis

The statistical difference between controls and patients regarding motor threshold, silent period duration and CCT was determined by using a Student’s t test. Values of P < 0.05 were considered to be significantly abnormal. For the I-waves summation, as the sample size did not allow a Gaussian distribution of data, we used non-parametric tests such as the two sample Mann–Whitney U test. The level of significance was set at P < 0.05.

3. Results

3.1. Normal subjects

In FDI muscle, significant I-waves facilitation with MEPs up to 4.5 times the algebraic sum of the single stimuli given alone occurred at 3 distinct peaks at ISIs of 1.5, 2.9 and 4.5 ms (Fig. 1).
3.2. ALS patients

The mean threshold intensity of MEP elicitation was significantly higher \((52.9 \pm 21)\) with respect to control values \((P < 0.02)\). A dramatic reduction of the mean silent period duration (obtained at 130\% of RMT intensity) was also recorded \((P < 0.001)\). The CCT, calculated by means of F-wave, was significantly prolonged \((P < 0.004)\). The I-waves interaction in ALS patients was evaluated by pooling out the data according to CCT. As plotted in Fig. 1 the ALS patients with abnormal CCT had a significant reduction of I-waves summation in the first peak. On the other hand, in patients with normal CCT, the I-waves summation was normal except in 6 patients in which the I-waves summation exceeded 2 SD. Statistical analysis considering the ISI of 1.5 ms, with respect to normal values, revealed a statistical significant reduction of I-waves summation for ALS patients with abnormal CCT \((P < 0.02)\). The I-waves summation did neither correlate with the disease duration nor with ALS functional rating scale (linear regression calculated by means of Pearson’s squared \(r\) test). The reduction of I-waves summation was statistically significant also at ISI of 2.9 and 4.5 ms \((P < 0.01)\). This was true for both patients with normal and abnormal CCT.

In Fig. 2 we plotted the distribution of the first peak of facilitation at 1.5 ms with respect to 95\% of upper and lower confidence limits of normal values. MEP size, after paired stimulation at 1.5 ms ISI, is expressed as a percentage of the sum of S1 + S2. It is interesting to note that at least 6 patients with normal CCT showed an I-waves summation, which exceeded 2 SD of normal values. The two horizontal dashed lines refer to 95\% of upper and lower confidence limits of normal values.

The 6 patients, with an I-waves summation which exceeded +2 SD of normal values, were re-tested after 1 year. The clinical picture was different compared with the baseline: the deep tendon reflexes were absent and there was a marked muscle hypotrophy, especially in hand and extensor muscles. In Fig. 3 we plotted the amount of I-waves facilitation at the baseline and also 1 year later.

It is interesting to note that a year later there was a substantial decrease in the amount of facilitation in all peaks. Decrease in I-waves facilitation in the same patients was probably caused by the degeneration of cortico-spinal

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**Fig. 1.** Short latency facilitation between pairs of threshold magnetic stimuli applied to human motor cortex in normal subjects and in ALS patients. Averaged motor responses in the relaxed FDI of 10 controls (circles), 12 ALS patients with normal CCT (squares) and 13 ALS patients with abnormal CCT (triangles), are plotted against the inter-stimulus interval. MEP size, after paired stimulation, is expressed as a percentage of the sum of S1 + S2 (see Section 2). The intensity of S1 and S2 was fixed at resting motor threshold level.

**Fig. 2.** Distribution of the first peak of facilitation at 1.5 ms with respect to the 95\% of upper and lower confidence limits of normal values. MEP size, after paired stimulation at 1.5 ms ISI, is expressed as a percentage of the sum of S1 + S2. It is interesting to note that at least 6 patients with normal CCT showed an I-waves summation, which exceeded 2 SD of normal values. The two horizontal dashed lines refer to 95\% of upper and lower confidence limits of normal values.

**Fig. 3.** Facilitatory I-waves interaction in the motor cortex of ALS patients: follow-up after 1 year. Six patients, with I-waves summation exceeding 2 SD of normal values, were re-tested after 1 year. Averaged motor responses in the relaxed FDI at baseline (squares) and 12 months later (circles) are plotted against the inter-stimulus interval. MEP size after paired stimulation is expressed as a percentage of the sum of S1 + S2.
tract. Furthermore, the patients were pooled out in two groups: the first group containing patients in which I-waves summation exceeded 2 SD and a second group with patients in which I-waves summation was below 2 SD. The disease duration in these two groups was completely different with a much shorter duration in patients with a hyperexcitable pattern of I-waves summation $P < 0.001$ (Fig. 4). The threshold was significantly lower in patients with enhanced I-waves summation compared to patients with reduced I-waves summation ($P < 0.001$). The CSP was not statistically different between these two groups ($P < 0.4$). The raw data of RMT, AMT, CSP, I-waves summation and disease duration are summarised in Tables 1 and 2.

### 4. Discussion

The main finding of our study is an alteration of I-waves summation in ALS patients that affect all 3 peaks. All the patients were treated with riluzole, which is a drug with anti-glutamatergic action, which might affect cortical excitability thus interfering with I-waves summation. However, it has been shown that riluzole does not interfere with I-waves summation that is rather under strict GABA-ergic control (Ziemann et al., 1998b). In the following discussion we will refer to the first peak of facilitation, which is the best characterised. Experiments using either magnetic followed by anodal electrical stimulation or pair of anodal electrical stimuli, suggested that the first peak of I-waves facilitation most likely occurs within the motor cortex (Tokimura et al., 1996). Recent epidural spinal recordings made in conscious humans, in the absence of anaesthetic agents that suppress the generation of I-waves, have confirmed the cortical origin of this first peak (Di Lazzaro et al., 1999).

There is one puzzling question about the nature of this first facilitatory peak. Which neural elements are excited by the second stimulus? One possibility is that the first stimulus raises the excitability of some elements, without reaching their threshold for producing an action potential. It might be possible that the first stimulus depolarises regions such as the initial axonal segment or the membranes of the cell body, even if it is insufficient to trigger neuronal discharge (Amassian et al., 1998; Di Lazzaro et al., 1999). In such cases the second shock might be able to recruit these cells to fire and the resulting cortico-spinal volley would be larger than expected. Possible candidate regions for this effect would be axonal hillock, at the pyramidal neurons themselves, or cortical inter-neurons of layers II and III and experiments so far have suggested this. (Nakamura et al., 1996; Ziemann et al., 1998a). The pattern of alteration of the first facilitatory I-waves peak seems to be different according to the CCT, which is the widely accepted marker for the relative integrity of the cortico-spinal tract. In patients with inexcitability of cortical motor pathways and prolongation of CCT, the reduction of the first peak of I-waves summation might reflect a functional impairment or a loss of the cortico-motoneuronal projections converging on the spinal motoneurons (SMN) under study. However, it might also be

### Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>RMT</th>
<th>AMT</th>
<th>CSP</th>
<th>I-waves (1.5 ms)</th>
<th>Duration of disease (months)</th>
<th>Dominant clinical findings</th>
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<td>1</td>
<td>35</td>
<td>28</td>
<td>40</td>
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<td>40</td>
<td>35</td>
<td>50</td>
<td>1100</td>
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<td>32</td>
<td>38</td>
<td>1145</td>
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<td>25</td>
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</table>

*Clinical and neurophysiological data in patients with normal CCT.*
argued that a reduced excitability of SMN, especially in later stage of the disease, would preclude full facilitation by the descending cortico-spinal volley and might therefore be responsible for the lack of I-waves summation. There are evidences that in ALS the excitability of spared SMN may be normal. Eisen et al. using post-stimulus histogram (PSTH) studies showed a normal generation of excitatory post-synaptic potentials (EPSP) in ALS patients (see subsequently) at least early in the disease. In this case, which are the mechanisms responsible for the reduction in the first of peak I-waves summation in ALS? In the brain of ALS patients there is a loss of pyramidal and Betz cells in the motor cortex, with foci of gliosis in layers II and III and deep subcortical white matter junction (Chou, 1992).

Considering that the I-waves summation in the first peak occur at sites such as the axonal hillock of the pyramidal neurons or cortical inter-neurons of layers II and III it is reasonable to assume that the neuronal degeneration in ALS would preclude a normal I-waves summation within the motor cortex. Six out of 11 patients with normal CCT showed a marked facilitation of the first peak. In the same patients single pulse TMS studies have shown a reduced motor threshold. Our data thus suggest that, at least in a minority of patients with ALS, there is a hyperexcitability of upper motoneurons. A number of neuroimaging and neurophysiologic studies support the hypothesis that in ALS the motor cortex may be hyperexcitable. As assessed by PET, during unilateral hand movements, ALS patients show significantly greater increases of metabolic activity than control subjects in the hand/arm area of the primary sensory motor cortex bilaterally, in the face area contralaterally and in the contralateral premotor and supplementary motor cortices (Abrahams et al., 1996).

A variety of TMS experiments also indicate that the ALS motor cortex may be hyperexcitable. The resting motor threshold is frequently lowered (Eisen et al., 1990; Desiato and Caramia, 1997), the cortical silent period is shortened (Prout and Eisen, 1994) and intra-cortical inhibition at short ISIs (<4 ms) is reduced in ALS (Yokota et al., 1996; Ziemann et al., 1997).

Enhanced excitation or reduced inhibition could both be key factors in the development of hyper-excitability of the cortico-motoneurons. The evidence for increased accumulation of glutamate excitatory transmitter, which could stimulate the cortico-motoneurons to discharge excessively, is compelling (Shaw, 1994; Rothstein, 1995). Moreover, recent clinical trials have shown that anti-glutamate agents can slow the progression of ALS in both transgenic mice model of familial ALS (Rothstein, 1995) and in humans (Bensimon et al., 1994). However, equally, cortical hyper-excitability in ALS could be secondary to failure of local circuit inhibitory inter-neurons to properly modulate cortico-motoneuronal firing. GABA is the most powerful inhibitory transmitter in the nervous system and about 10% of all neocortical neurons are GABA accumulating local circuit neurons (Deisz and Luhmann, 1995). GABA receptors are even present on excitatory terminals and diffusion of GABA to adjacent glutamatergic terminals curtails the release of excitatory aminoacids (Thompson et al., 1993). Considering that the production of I-waves is primarily controlled by GABA related neuronal circuits it is likely that the increased facilitation of the first peak may be induced by the selective loss of GABAergic inter-neurons in ALS motor cortex. It is interesting that the 6 patients with a hyperexcitable pattern of I-waves summation who were re-tested after 1 year showed a clear reduction of facilitation. These findings could indicate that there is a continuum of abnormalities in the early and late stages of the disease. This is also supported by the fact that the disease duration was shorter in patients with enhanced I-waves summation than in patients with reduced I-waves summation. This reduction was statistically significant also at ISI of 2.9 and 4.5 ms ($P < 0.01$). This was true for patients both with normal or abnormal CCT. The exact nature of the facilita-
tory I-waves interaction at 3 and 4–5 ms is not well established. Given that such intervals are close to the known periodicity of I-waves, it is tempting to suggest that the first magnetic stimulus might initiate activity in circuits involved in I-waves generation and evoke a series of EPSPs in cortico-spinal neurons. If excitatory input from the second stimulus coincided with this, then cortical output would be enhanced and EMG responses facilitated. However, this interaction could also occur at subcortical levels (Tokimura et al., 1996; Ziemann et al., 1998a). It is intriguing to note that the I-waves summation of the II and III peak was reduced in both ALS patients with normal or abnormal CCT.

We can speculate that a loss of periodicity of I-waves might account for this reduction of I-waves summation at 3–5 ms intervals. However, a spinal cord involvement cannot be excluded especially in the late stage of the disease. The findings of the present article are partially in keeping with those of Salerno and Georgescu. One major point of difference is the presence of a normal or even facilitatory pattern of I-waves summation in ALS patients with normal conduction time. However, it is likely that the patients studied by Salerno and Georgescu, considering their high motor threshold, were tested in a later stage of the disease with a clear alteration of the CCT.

4.1. Comparison with PSTH studies

Some insight into the behaviour of I-waves can also be obtained from single motor unit recordings using needle electromyography (peri-stimulus time histograms or PSTHs). These studies provide information regarding the synaptic input to single SMNs and have demonstrated that they receive a sequence of EPSPs consistent with the arrival of multiple monosynaptic from D and I-waves volleys (Day et al., 1987, 1990).

The results of our study are in keeping with the data reported by Eisen et al. (1996). In this article PSTHs of a discharging single motor units, during TMS, were obtained in ALS patients and in controls. Normal subjects, as reported in literature (Day et al., 1987), had an early period of increased firing probability of motor unit occurring at about 20 ms post-stimulus, reflecting an underlying compound EPSP induced by fast-conducting, descending volleys of the cortico-motoneuronal core facilitating the single SMN. Compared with age-matched controls, the EPSP in most patients with ALS were reduced in amplitude (Eisen et al., 1996). These data suggest again that in ALS a reduction of the cortico-motoneuronal core projecting on the spinal motor neurons is responsible for the lack of I-waves summation. In the same study patients in the early stages of the disease showed the presence of large EPSP in PSTH studies after TMS which might account for the increase of facilitation of the fist peak found in our study.

Although the PSTHs studies provide valuable information on the physiology of I-waves it must be taken into account that the cortico-spinal volley also activate other inter-neurons in the spinal cord, such as the Ia inhibitory inter-neurons, which then project to SMNs. The consequence is that cortico-spinal activity can result in a sequence of EPSP inhibitory post-synaptic potentials (IPSPs) at the motoneurons (Cowan et al., 1986).

In conclusion, ALS patients showed two different and opposite patterns of I-waves summation. It could be hypothesized that the hyper-excitability of motor cortex is present in an earlier stage of the disease when the cortico-spinal tracts are almost intact (normal CCT) but there is a loss of GABA-ergic inter-neurons with a glutamatergic overactivity. On the other hand in the later stage of the disease there is a reduction of I-waves summation when the glutamate toxicity has caused a substantial reduction in the cortico-spinal projections converging on the SMNs.

References


