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The effect of riluzole in amyotrophic lateral sclerosis: a study with cortical stimulation

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Abstract

A population of 31 patients with sporadic amyotrophic lateral sclerosis (ALS) was selected for a prospective open study based on treatment with riluzole. A neurophysiological evaluation was performed by means of single and paired transcranial magnetic stimulation (TMS). The examined parameters, excitability threshold, motor evoked potential (MEP) duration, silent period (SP) duration and time course of intracortical inhibition to paired TMS after 6 months treatment, were matched against those recorded from the patients themselves before the beginning of treatment and from 20 (single TMS) or 10 (paired TMS) age-matched control subjects. Normal behaviour of the SP in response to increasing TMS was found in the treated patients; they showed a significant linear correlation between these two parameters ($r = 0.96$) comparable to that calculated for controls ($r = 0.98$), and significantly different with respect to drug-free patients ($r = 0.8$, $P = 0.014$). A significant reduced size of the 'conditioned' MEPs to paired stimulation was documented in the treated patients compared with the untreated patients ($P = 0.002$). Our neurophysiological contribution to the assessment of the effect of riluzole on the motor cortical inhibitory property in ALS may be considered a setting for controlled trials in extended patient series, even in a pre-clinical phase.

Keywords: Amyotrophic lateral sclerosis; Motor evoked potential; Silent period; Paired magnetic stimulation; Riluzole

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder which primarily affects motor cortical and brain stem/spinal grey matter. The central disturbance of motor control leads to impaired activation and to abnormal recruitment of alpha motor neurones [1]. Hypothesised pathogenetic mechanisms consider ALS primarily a disease of the cortico-motor neuronal system with secondary trans-synaptic spinal motorneuron degeneration [2,3]. One of the most recent assumptions about the etiology holds that glutamate, the excitatory neurotransmitter in the brain, accumulates to toxic concentrations at synapses, due to a severe loss of transport on neurones and astroglial cells [4–6]. Two main mechanisms have been envisaged: (a) inactivation of voltage-dependent neuronal and glial sodium channels; (b) non-competitive blocking of excitatory amino acid receptors and/or stimulation of a G-protein-dependent process, promoting both inhibition of glutamic acid release and blockade of post-synaptic events mediated by activation of NMDA receptors [7]. However, it has not yet been established whether the abnormalities in the glutamate system reflect the primary or the only cause of the disease.

Riluzole (2-amino-6-difluoromethoxy benzothiazole) is a glutamate antagonist investigated in the rat neocortex for its effect on cortico-cortical excitatory synaptic transmission through the inhibition of cortical field potentials (mainly dependent on the activation of non-NMDA glutamate receptors) evoked by intracortical electrical stimulation [5]. This property, likely mediated by both voltage-activated sodium and calcium channels, initially suggested the possibility of treating epileptic diseases and later neurodegenerative diseases [8].

On both experimental and clinical bases the employment of treatment with neuroprotective agents capable of de-

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creasing neuronal hyperexcitability appears rational. In fact, the reduced inhibitory power of the motor cortex could be counteracted by antiglutamate drugs, which decrease neuronal hyper-excitability. The neurotoxicity secondary to excitatory mechanisms can be reduced by the chronic inhibition of glutamate uptake upon neurons and astroglial cells [9]. The employment of riluzole in ALS has shown encouraging results in prolonging survival and time to tracheostomy in patients suffering from ALS [10–12].

The neurophysiological correlates of cortical changes in ALS documented by transcranial magnetic stimulation (TMS) are: increased excitability and reduced inhibition, corresponding to a lowered threshold for MEP elicitation; shortened duration of the cortical silent period (SP) with respect to normal (the reduction to absence of the SP can be considered a neurophysiological marker of this disease); absence of intracortical inhibition normally occurring in response to paired stimulation, whilst the motor central conduction time is reported to be a poor indicator of diagnosis and prognosis in ALS [13–19].

In this study we investigated whether or not the excitability changes tested by means of TMS (the SP duration and the intracortical time course of motor inhibition to paired cortical stimulation) can be modified, towards normal values, by riluzole administration. In order to verify this hypothesis, we examined and compared two groups of ALS patients: 'treated' versus 'drug-free' patients, both matched against healthy controls.

2. Subjects and methods

2.1. Patients

Thirty-one of 49 patients, suffering from the sporadic form of ALS, were selected for a longitudinal open-label prospective study based on treatment with riluzole. The eligibility of patients was assessed according the same criteria established in two previous studies performed on ALS patients in treatment with riluzole [10,11]: (i) clinical history shorter than 5 years; (ii) no detection of conduction blocks in peripheral nerve conduction velocity; (iii) no paraproteinemia on serum immuno-electrophoresis; (iv) no dementia. The patients exhibited a probable ($n = 7$ patients) and a definite ($n = 24$ patients) form of the disease according the 'El Escorial' diagnostic criteria [20]. The mean time interval between the onset of symptoms and the beginning of treatment was 17.3±12.1 (5–48) months. The limbs were first affected in 21 patients, whilst the disease onset was bulbar in 10 (Table 1). Among the diagnostic tests performed, neither multifocal motor conduction blocks in the course of the neurophysiological examination, nor sera anti-GM1 ganglioside antibodies, were found. The patients' neurophysiological data were matched against those of a control population represented by 20 drug-free healthy volunteers (13 male, eight female). Patients and control subjects were statistically comparable for age (58.5±11.9 years vs. 60.8±13.9 years, respectively). Oral treatment with riluzole (Rilutek®, 50 mg, one tablet, bid.) was directly supplied by Rhone-Poulenc-Rorer® (Origgio, Varese, Italy) during the first part (12 months) of the longitudinal open-label study after the approval of the Health Institution. The mean duration of the follow-up was 10.6±8 (2–33) months.

2.2. Clinical evaluation

Muscle strength was quantified by means of the Medical Research Council (MRC) muscle power rating scale. The clinical score according to Norris was calculated during the evaluation performed in all patients after each neurophysiological procedure [21]. The pharmacological, as well as the neurophysiological procedures were performed after written informed consent was obtained from both patients and healthy subjects and approval of the Local Ethical Committee.

2.3. Stimulation and recording procedures

The TMS investigation included two protocols: (1) the evaluation of motor evoked potentials (MEPs) and SP parameters, recorded in response to single magnetic stimulation; (2) the evaluation of the time course of intracortical motor activity tested with pairs of magnetic stimuli applied at inter stimulus intervals (ISIs) of 1–6 ms.

2.3.1. TMS

MEPs were recorded from intrinsic hand muscles (abductor pollicis brevis (ABP), first dorsal interosseus (FDI)) of both sides (single TMS) and of the less affected side (double TMS) via surface electrodes applied in a belly-tendon montage. Patients exhibiting severe atrophy and weakness of the target muscles (Medical Research Council (MRC) 3/5 or less) were discarded from the protocol.

Table 1
Clinical features of patient population

Sex (%)		Age (years)		Diagnosis (%)		Clinical signs at onset (%)	
Male	Female	Mean	SD	Probable	Definite	Limbs	Bulbar
14 (45.1)	17 (54.8)	58.5	11.9	7 (22.5)	24 (77.4)	21 (67.7)	10 (32.2)

2.3.2. Single TMS

The stimulation was applied using a high-power Magstim 200 magnetic stimulator (Magstim, Whitland, Dyfed, UK) connected to a circular regular coil (13 cm outer diameter) applied to the scalp region overlying the central sulcus for the stimulation of the hand motor area. With the Magstim monophasic pulse generator, optimal excitation was obtained with the coil orientation side 'B up' for the left hemisphere and side 'A up' for the right hemisphere.

Signals were amplified and filtered between 2 and 2000 Hz (−6 dB/oct roll-off, ESAOTE-Multibasis). Three responses were averaged in the 100–500 ms interval following each trans-cranial stimulus for each trial. MEPs were recorded during relaxation of the target muscle for the threshold measurement [22,23], whilst a moderate contraction (20% of maximal force, as measured with a force transducer) allowed the detection of both MEP and SP characteristics in the 500 ms following TMS. Brain stimuli were presented at a rate of 0.16 Hz, in order to avoid fatigue and possible habituation of the brain in producing evoked responses. Trials were repeated at least once in order to detect reproducibility of the MEPs. The stimulation was then progressively increased in steps of 5%, up to 100%, with the aim of assessing parallel SP duration increments.

2.3.3. Paired (double pulse) TMS

A figure-of-eight coil was connected to two Magstim units through a Bi-stim module. The coil was placed over the central sulcus, on the scalp region corresponding to the hand motor area. The focal coil was held tangential to the skull with the handle pointing backward and at 45° lateral from the midline. The site where MEPs with the lowest intensity ('threshold') were elicited in the contralateral, constantly relaxed, target muscle was carefully located, and the subject or patient was required to maintain alertness with open eyes, in order to optimise the MEP's elicitation [24]. A conditioning-test design was used for investigating the time course of MEP inhibition. Paired stimuli were applied with conditioning pulses delivered 1–6 ms before test stimulation [19,25,26].

The intensity of the conditioning pulses was maintained below the threshold for evoking responses in contracted muscles, while test pulses were delivered just suprathreshold for eliciting relaxed MEPs, the difference in intensity between test and conditioning stimuli amounting to about 40% of the device's maximal output. Because thresholds could fluctuate during a session, the basic stimulating parameters were randomly checked and, when necessary, adjusted in order to maintain a stable level of recording.

Recordings were acquired with a double amplification so that saturated responses were measurable on-line with suitable calibration. In each set of experiments, test and conditioning pulses at the different ISIs were randomly intermixed. Several blocks of trials were performed in order to achieve a complete set of ISIs. Each block included eight trials with two options: the MEP in response to the test stimulus alone and the MEP conditioned by a prior sub-threshold stimulus delivered at one of the pre-settled intervals. The sequence began and ended with two 'test only' trials with the conditioned MEPs occurring in between (Fig. 1). Paired TMS could be performed in 10 of 31 patients enrolled in this study, due to the increased threshold required for evoking relaxed MEPs, exhibited by

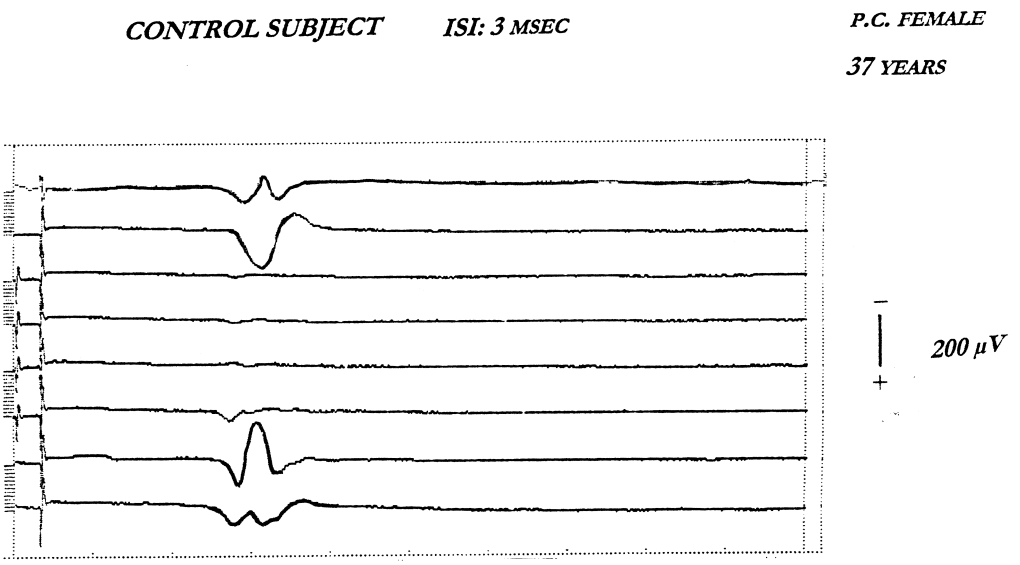

Fig. 1. Paired TMS, performed in a control subject. MEPs to focal TMS in relaxed thenar muscles are inhibited by a prior sub-threshold conditioning stimulus at an ISI of 3 ms. The top two and lower two traces show the response to the test stimulus alone, whilst the middle four traces represent the conditioned (missing) MEPs. Analysis time 10 ms/div.

19 patients, which was too high for the Bi-stim module device. In this part of the study, the normative values were obtained from 10 healthy volunteers.

2.3.4. Temporal profile of neurophysiological evaluation

A retrospective analysis of the neurophysiological examination (single TMS) was performed in eight patients, who were investigated and monitored over the 12 month period preceding the beginning of riluzole treatment in our laboratory. In the single TMS protocol, for each treated patient, the SP mean duration was measured over time at 80–100% intensity, at 1, 3 and 6 months and at the latest time of follow-up. In addition, the mean SP duration in all treated patients at threshold and at 5% steps of increasing TMS intensity, measured after 6 months of treatment, was matched against the corresponding values of TMS recorded from the same patients tested just before treatment initiation, and against those of controls. In eight patients an additional evaluation was made within 12 months of treatment. The timing of paired TMS covered neurophysiological sessions performed after 1, 3 and 6 months from the beginning of riluzole treatment. The control subjects underwent both TMS protocols in a unique recording session.

2.4. Data analysis

The following parameters were measured and analysed:

2.4.1. Single TMS

1. Excitability threshold, expressed as percentage of stimulator maximal output (%);
2. SP duration (ms), obtained at threshold and at increasing values of stimulus intensity, measured from the end of MEP (latency plus duration) up to the rebound of voluntary EMG activity, with 0.5 mV gain sensitivity and analysis time ranging from 200 to 500 ms;
3. Duration of 'contracted' MEPs (ms).

2.4.2. Paired TMS

1. Amplitudes (peak-to-peak, μV) of both 'test' and 'conditioned' MEPs and their differences measured as percentages of control size (conditioned MEP = % of test MEP).

The statistical comparisons were performed between patients and control subjects, as well as between treated and untreated patients.

2.4.3. Single TMS

1. Means/standard deviations (SDs), cross-correlation of all the examined parameters via paired and unpaired Student's *t*-test (a probability of 0.05 or less accepted as significant);
2. Correlation between variables calculated through linear regression analyses, with r^2 values referring to the multiple squared Pearson correlation coefficients: for each condition (treated patients, untreated patients and control subjects), the SP duration (dependent variable) was related to the corresponding increase in TCS values (independent variable). Comparisons between linear regression coefficients were obtained by a two-tailed Student's *t*-test, using a confidence level of 95%.

2.4.4. Paired TMS

A multiple analysis of variance (MANOVA) was performed on the time course data (conditioned MEP size, expressed as percentage of test MEP) at each ISI interval for the three conditions (untreated patients vs. control subjects, treated vs. untreated patients, treated patients vs. control subjects). The Greenhouse–Gaisser (G&G) correction was utilised when more than two 'levels' were presented in a 'within' factor. The design of a two-way ANOVA was used to compare the patients versus control subjects, with group as 'between' factor with two levels (control subjects vs. patients) and ISIs as 'within' factor with six levels (1–6 ms ISIs). A two-way ANOVA analysis for repeated measures was utilised for comparing conditioned MEPs in the patient groups with two 'within' factors: (i) treatment, with two levels (before and after riluzole); (ii) ISIs, with the six levels reported above. When a significant interaction between factors was found, the singular differences between means were assessed via the post-hoc Tukey Honest test for unequal sample size. All statistics were performed with the CSS/3 program.

3. Results

3.1. Drop out clinical evaluation

In one 56-year-old patient the riluzole treatment was interrupted after 8 weeks, due to an increase of serum liver enzymes (four-fold normal values of transaminases) recorded at the end of the first and second month.

The mean Norris score was comparable in each evaluation session for treated patients (baseline, 67.1±21; 1 month, 77.2±16.7; 3 months, 76.6±18.7; 6 months, 73±20.5), whilst the MRC score was significantly higher than the baseline value when calculated at 1, 3 and 6 months.

3.2. Single TMS

The principal finding observed in this part of the study is represented by a prolongation of the SP duration measured during the course of treatment, showing a peak of effect at the third month, compared to baseline values scored before treatment. Mean values and standard deviations (SDs) of the examined parameters are listed in Table 2. The statistical evaluation of the SP to increasing TMS is reported in Table 3.

Table 2
Neurophysiological results for the single TMS protocol

Parameter	Untreated patients	Treated patients[a]	Control subjects	Statistical evaluation
Threshold (%)	60.7±18*	66±19*	45.8±4.6	Mean±SD
MEP duration (ms)	28.6±9*	27.8±8.5*	23.7±5.4	Mean±SD
SP duration (ms)	57.8±23.9*	86.8±35.6	114.6±58.5	Mean±SD

[a] At the sixth month.
Comparison between patients and control subjects: $*P < 0.001$.

Table 3
Statistical analysis of the single TMS protocol

Parameter	Treated patients		Untreated patients		Parameter
SP mean duration (ms)	86.81±35.6		57.83±23.9		$P = 0.01$
SP duration to increasing TMS	$r = 0.96$	$z = 2.56$	$r = 0.9$	$z = 2.1$	$n = 15$
	Treated patients		Control subjects		
SP mean duration (ms)	86.81±35.6		114.6±58.5		$P = 0.12$
SP duration to increasing TMS	$r = 0.96$	$z = 2.56$	$r = 0.99$	$z = 3.7$	$n = 15$
	Untreated patients		Control subjects		
SP mean duration (ms)	57.8±23.9		114.6±58.5		$P = 0.002$
SP duration to increasing TMS	$r = 0.9$	$z = 2.2$	$r = 0.99$	$z = 3.9$	$n = 15$

3.2.1. Evaluation at the sixth month

In the drug-free patients, the regression coefficients, calculated between increasing values of TMS and SP duration, did not show the same slope inclination as found in the control subjects. A marked reduction of SP prolongation to increasing TMS was found in these patients vs. control subjects (control subjects, $r = 0.98$; patients, $r = 0.8$; $P = 0.002$; Fig. 2).

By contrast, in treated patients a significant regression coefficient between TMS intensity and SP duration was found ($r = 0.96$). The statistical comparison of the correlation coefficients documented a significant difference between the two patient groups (treated vs. untreated, $P = 0.014$; Fig. 3). No significant difference of SP prolongation to increasing TMS intensities between riluzole-treated patients and control subjects was observed ($P = 0.12$).

In eight patients analysed retrospectively (within 12 and 24 months preceding treatment), a progressive reduction of SP duration was detected, both at low range (<50%) and at high range (80–100%) of TMS. After riluzole treatment, the SP prolongation measured in the high range of TMS intensity showed no modification of its mean values over

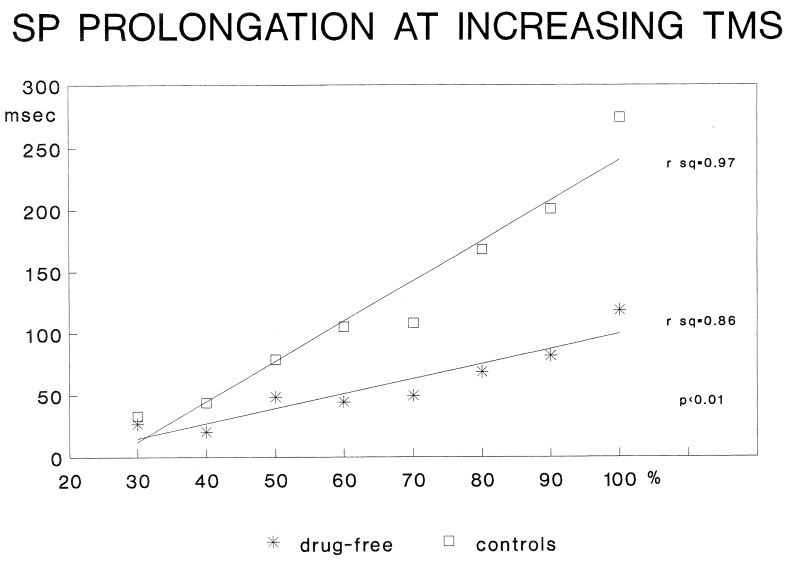

Fig. 2. The regression lines show significant divergence between untreated 'drug-free' patients versus controls.

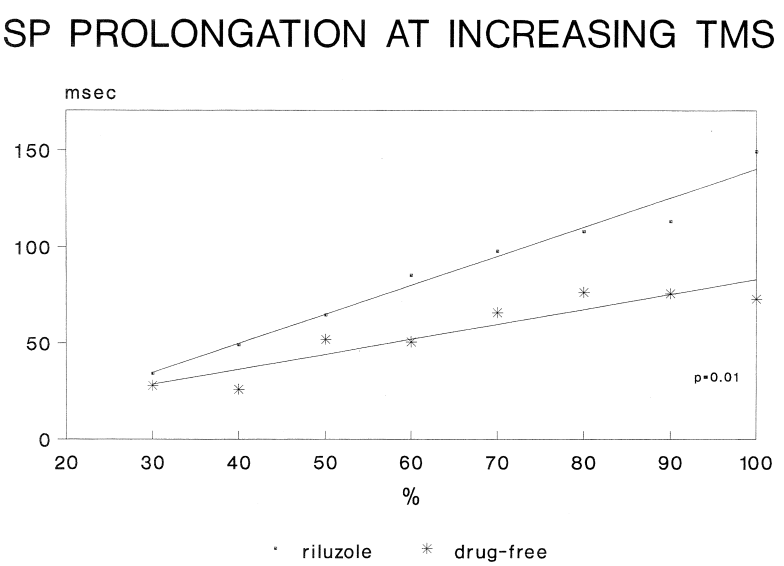

Fig. 3. The regression lines show significant divergence between untreated patients versus treated patients.

the 12 months of follow-up in 12 of 31 patients (Fig. 4). In four patients, having respectively 33, 18, 96 and 10 months of clinical history, no MEP could be elicited from intrinsic hand muscles even at 100% TMS intensity, whilst detectable MEP-SP sequences were still evoked from forearm muscles at high intensity of TMS. No significant changes in both excitability threshold and MEP duration resulted over the first 6 months of treatment compared to the values measured before treatment (Table 2, $P = 0.9$ and $P = 0.1$, respectively). Mean excitability threshold and MEP duration values were higher for patients (both treated and untreated) than for controls.

3.3. Paired TMS

The mean clinical history of the patients tested with double TMS was 20.1±10.6 months, comparable to that of ineligible patients (22.6±26 months, $P = 0.35$). The time course of intracortical inhibition exhibited by the three groups is reported in Fig. 5 and Table 4.

3.3.1. Comparison between 'drug-free' patients (≠9) and control subjects (≠10)

The 'group' factor showed a significant ($F = 10.24$, $P = 0.005$) effect, because the conditioned MEP's size was significantly less inhibited in patients (106%) than in control subjects (46.6%). The 'ISI' factor was also significant ($F = 8.25$, $P < 0.001$), because a facilitatory effect was observed at an ISI of 5 and 6 ms (106% and 103.5%, respectively), whereas at 1–4 ms ISIs a time course of inhibition was found, ranging from 47.9 to 89.7%. Finally, the interaction between the two factors ('group'×'ISI') was also significant ($F = 3.52$, $P = 0.02$), as explained by the post-hoc Tukey Honest test showing, in the 1–4 ms ISI ranges, conditioned MEPs less inhibited in patients than in control subjects ($P = 0.006$, $P = 0.0001$, $P = 0.02$ and $P = 0.03$ for 1, 2, 3 and 4 ms, respectively; Fig. 6).

3.3.2. Comparison between patients before and after treatment (≠9)

The ANOVA two-way analysis showed a significant ($F = 4.9$, $P < 0.05$) effect of the factor 'treatment' (drug-free vs. riluzole), because riluzole increased the MEP inhibition in patients from 106.6 to 65.4%. The 'ISI' factor was also significant ($F = 6.47$, $P < 0.01$), as the percentage increased from 55.8 to 121.3% as the ISI increased. Finally, the 'treatment'×'ISI' interaction showed a significant effect ($F = 4.34$, $P = 0.002$). In the treated patients, the post-hoc Tukey Honest test displayed the most significant recovery of MEP inhibition at 4 ms ISI (123% vs. 42% pre- and post-riluzole, respectively; $P = 0.006$).

3.3.3. Comparison between control subjects (≠10) and treated patients (≠9)

The ANOVA two-way analysis of conditioned MEP response (corrected by the G&G method) showed no significant effect of the 'group' factor, because the mean of the conditioned MEP amplitude in normal subjects (46.6%) was not significantly different from that observed in treated patients (64%). The factor 'ISI' was significant ($F = 29.7$, $P = 0.0001$), because the facilitatory effect of the conditioning stimulus increased across all the ISIs from 17 to 120%. The interaction between the two factors ('group'×'ISI') was not significant because both groups had the same trend of behaviour at any given ISI.

4. Discussion

TMS has proven to be a suitable technique for both diagnosing and monitoring ALS progression; it can reveal

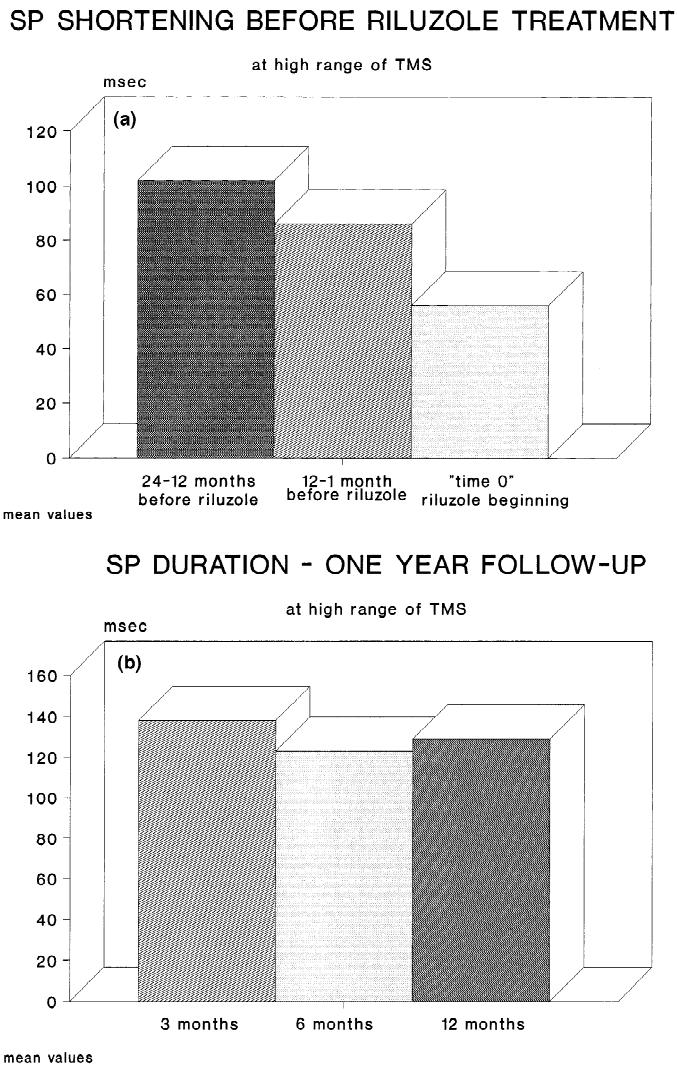

Fig. 4. Temporal analysis of SP duration, performed in eight patients over 24 months up to the beginning of riluzole treatment (time 0), measured in the high range of TMS (80–100%).

a reduced excitability threshold in early stages, marked desynchronization of MEPs and shortened cortical SP duration throughout the course of the disease. The SP, which is normally positively correlated to TMS, mainly reflects a cortical origin [34], and fails to exhibit the normal prolongation to increasing TMS in ALS patients [13,18,27].

Physiopathological features of ALS might be, at least in part, the effect of excitotoxicity mechanisms, critical steps in the cascade of events leading to cell death.

Our study was based on a comparison of the neurophysiological status more than the progression of the disease in treated versus untreated patients, according to the objective of enrolling all the eligible patients. Nonetheless, in eight patients, retrospectively examined within 12 and 24 months preceding the treatment, the parameters tested by single TMS documented a progressive reduction of SP duration. The silent period prolongation to increasing magnetic brain stimuli, measured in our treated patients, suggests a partial cortical inhibitory recovery. This improvement, best evaluated in the sixth month after the beginning of treatment, exhibited its nadir (in terms of SP duration to a high range of TMS values) at the third month, but was still significantly present after 12 months. The increased SP duration in our patients was not coupled with coexisting more synchronised MEPs. This could suggest that we mainly measured the cortical, rather than the spinal, effects of riluzole on motoneurons. Due to the possible reduced excitation exerted by the motor cortical circuits, we could also measure SP duration when preceding excitatory responses (MEPs) were not detected. This feature strengthens the hypothesis that, in humans, the neuronal circuits involved in producing SP are quite distinct from, and have a threshold of TCS lower than, that required to obtain MEPs [28].

The second significant result of the present study is the

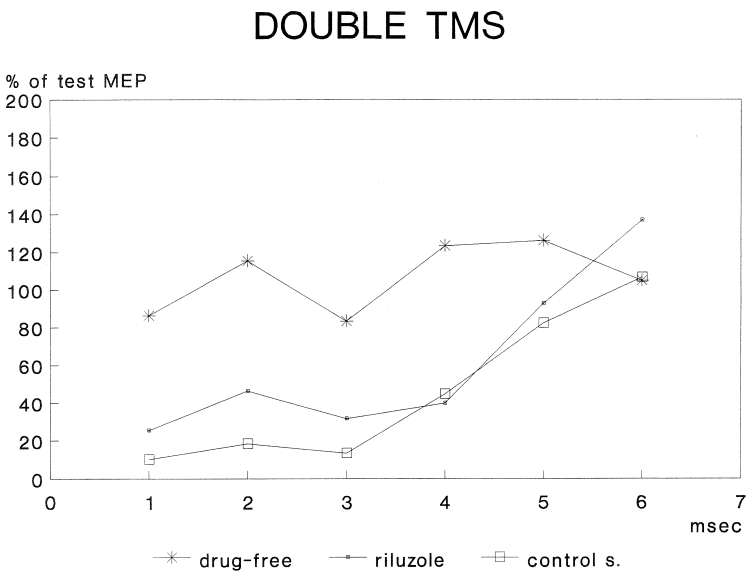

Fig. 5. Time course of intracortical inhibition in 10 control subjects and nine patients before and after 6 months riluzole treatment.

partial recovery of intracortical inhibition in the range 1–4 ms. Patients before treatment displayed an abnormal profile of intracortical motor activity: MEPs to test stimuli were not attenuated by preceding sub-threshold conditioning stimuli applied in the range 1–4 ms ISIs. By contrast, a significant reduced size of the conditioned responses was documented after 3 months of the beginning of treatment. The significant difference between pre- and post-riluzole recordings was found particularly at 4 ms ISI, although a significant difference was also present at 1, 2 and 3 ms ISI.

It should be noted that there was no significant inhibitory effect following riluzole after 3 weeks treatment, a finding which we observed in another study in progress (Caramia et al., in preparation), and which accords with the lack of acute effects of riluzole on motor unit parameters reported by Desai et al. [29], who observed that the therapeutic effect of riluzole is probably expressed clinically over time.

It has been assessed that inhibition tested with paired stimulation is settled by activation of intracortical, rather than spinal, mechanisms in healthy subjects. In fact, weak magnetic conditioning stimuli specifically engage cortico-cortical motor inhibitory circuits, having no significant influence on spinal motoneuron excitability [25,30]. In ALS patients such an intracortical mechanism is impaired [19,31]. In agreement with these reports, we have found a marked loss of the central motor inhibition to paired short-interval stimuli in our patients before treatment. The

Table 4
Time course of the conditioned MEPs expressed as percentage of the test MEPs (mean±SD)[a]

	ISI						Group mean
	1 ms	2 ms	3 ms	4 ms	5 ms	6 ms	
A. Normal subjects	9.5 ±5.4	11.3 ±6.3	14.2 ±7.4	56.1 ±43.2	86.5 ±41.5	102.2 ±52.5	46.6
B. SLA patients 'pre-therapy'	86.3 ±54.9	115.6 ±95.0	83.3 ±49.6	123.5 ±75.8	126.0 ±81.3	104.9 ±65.9	106.6** vs. A
C. SLA patients 'post-therapy'	25.4 ±13.9	53.6 ±18.6	41.2 ±12.6	42.2 ±23.6	95.6 ±41.9	137.9 ±57.9	65.4* vs. B
Means A+B ISI factor***	47.9	63.4	48.7	89.7	106.2	103.5	
Means B+C ISI factor**	55.8	84.5	62.2	82.8	109.3	121.3	
Means A+C ISI factor***	17.5	32.4	27.7	49.1	89.5	120.0	

[a] A vs. B, 'Group'×'ISI' interaction, $P = 0.02$. B vs. C, 'Treatment'×'ISI' interaction, $P = 0.003$. A vs. C, 'Group'×'ISI' interaction, n.s. Main factors: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

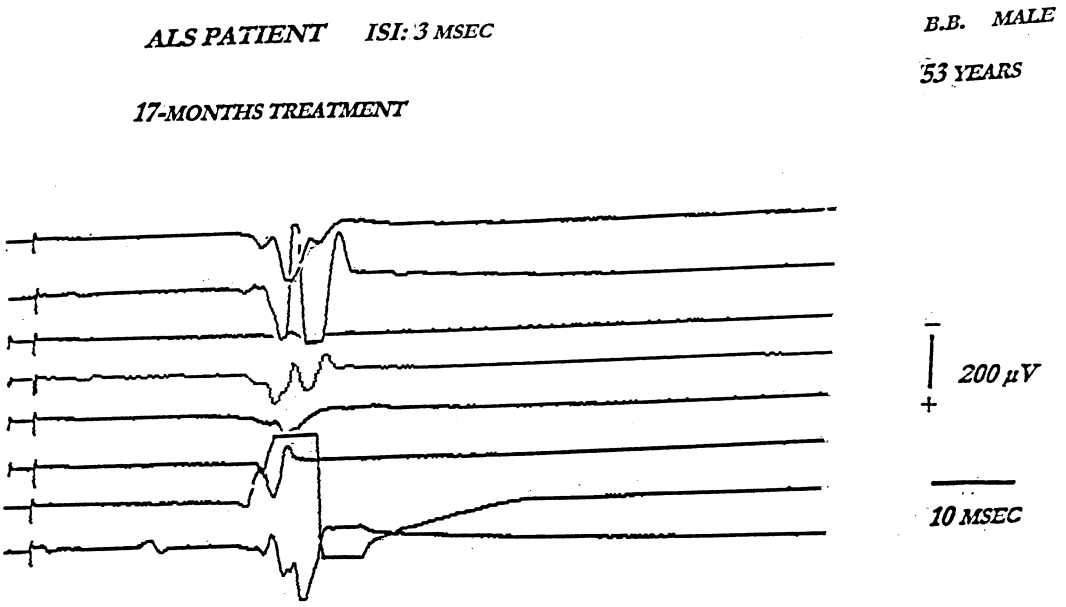

Fig. 6. Recordings from a patient obtained after 17 months of riluzole treatment, showing a marked inhibition of conditioned MEPs (four middle traces). ISI, 3 ms. Same construction as in Fig. 1.

reduced inhibitory power, documented by means of double TMS in sporadic ALS, has also been described in other brain diseases involving the grey matter, such as myoclonic epilepsy and cortico-basal degeneration [26,32]. On the other hand, the shortened silent period, as well as its lack of positive correlation to increasing TMS, has only been described, to our knowledge, in ALS disease.

Our results, as a neurophysiological contribution to the assessment of the influence of riluzole in treating ALS patients, can be adjoined to the clinical and instrumental findings documented in vivo [33]. The early diagnosis of this disease, as well as that of other cortical diseases, based upon the neurophysiological evaluation of specific parameters, calls for clinical controlled studies in extended patient series. In addition, the progression of clinical symptoms would require large and lengthy trials, in order to improve the impact of the results. Finally, detection of the preclinical phase is desirable. Since the biochemical cascade of events seems to offer different opportunities for therapeutic intervention, combination therapy may be the likely issue.

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