

Repetitive transcranial magnetic stimulation for ALS A preliminary controlled study

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Abstract

Repetitive transcranial magnetic stimulation (rTMS) of brain can modulate cortical neurotransmission, a novel paradigm of repetitive stimulation termed continuous theta-burst stimulation (cTBS) produces a pronounced and prolonged suppression of motor cortex excitability. The aim of this preliminary study was to investigate whether cTBS of motor cortex could have any beneficial effect in patients with amyotrophic lateral sclerosis (ALS). We performed a double-blind, placebo-controlled trial. Twenty patients with definite ALS were randomly allocated to blinded active or placebo stimulation. Repetitive stimulation of the motor cortex was performed for five consecutive days every month for six consecutive months. The primary outcome was the rate of decline as evaluated with the ALS functional rating scale. The treatment was well tolerated by the patients. Fifteen patients (seven active and eight sham) completed the study and were included in the 6-months analysis. Both active and sham patients deteriorated during treatment, however, active patients showed a modest but significant slowing of the deterioration rate. Though we cannot be sure whether the effects observed can be attributed to cTBS, because of the restricted number of patients studied, further investigation on a larger group of ALS patients is warranted. The results of the pilot study might open up a new therapeutic perspective in ALS based on neuromodulation. © 2006 Elsevier Ireland Ltd. All rights reserved.

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Amyotrophic lateral sclerosis (ALS) is a fatal disease with no cure. Glutamate-mediated excitotoxicity has been proposed as a possible cause of cell death in ALS [22]. Glutamatergic circuits of the human motor cortex can be activated non-invasively using transcranial magnetic stimulation (TMS) of the brain [8], and because the excitability of neural circuits in the cerebral cortex is not static, repetitive transcranial magnetic stimulation (rTMS) can produce changes in neurotransmission that outlast the period of stimulation [2,4,5,7,12,14,18,19,20,27].

Repetitive TMS has been already evaluated as a therapeutic tool in several neurological and psychiatric disorders, such as Parkinson's disease [17–24], chronic pain syndromes [13], dystonia [25], epilepsy [26,28] and depression [11] (see Wassermann and Lisanby 2001 for a review [30]). A recent review

suggested that the best effects of therapeutic rTMS are seen when protocols that depress network excitability are used to treat disorders characterised by cortical hyperexcitability [30].

In a proof of principle study, we found that motor cortex rTMS, at frequencies that decrease motor cortex excitability, causes a slight slowing in the rate of disease progression in ALS patients [9]. A tendency toward an increase in the rate of disease progression was observed in patients treated with rTMS at frequencies enhancing motor cortex excitability [9]. It has been suggested that the observed effects might be related to the rTMS induced changes in cortical excitatory neurotransmission and that any beneficial effect produced by rTMS protocols that reduce cortical excitability could be related to a diminution of glutamate-driven excitotoxicity [33]. A positive effect of motor cortex stimulation in ALS has also been suggested by a recent paper showing that invasive chronic motor cortex stimulation through subdural electrodes seems to reduce the rate of progression of the disease [23].

A recent study demonstrated that excitability of the motor cortex can be effectively reduced after application of a novel

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paradigm of repetitive transcranial magnetic stimulation termed continuous theta-burst stimulation (cTBS) [5,12]. Preliminary clinical observations in a patient with hemichorea suggest that cTBS might be a useful approach in the treatment of hyperkinetic disorders caused by motor cortex hyperexcitability [6].

The aim of this preliminary study was to investigate whether cycles of cTBS of the motor cortex can have any beneficial effect in ALS. The rationale for trying cTBS in this disorder is based on the evidence that it induces a long-lasting decrease of motor cortex excitability that could theoretically antagonize glutamate excitotoxicity in ALS by reducing the response of corticospinal cells to glutamatergic excitatory inputs. On the other hand, it has been also demonstrated that rTMS may modulate plasma levels of brain-derived neurotrophic factor (BDNF) in humans [1,31,32]. The BDNF is a potent survival factor for motoneurons that has been evaluated as a potential therapy for ALS. A neuroprotective effect of high-frequency rTMS is also suggested by a recent study using an experimental model of transient brain ischaemia in gerbils that demonstrated that the delivery of rTMS at high frequency 2–5 days before common carotid artery occlusion has a protective effect against delayed neuronal death of hippocampal neurons [10].

All the above preliminary observations support an evaluation of the effects of cTBS in ALS patients.

This preliminary study was approved by the ethics committee of the Medical Faculty of the Catholic University in Rome and patients gave their informed consent before participation. Eligible patients had a diagnosis of definite ALS according to the El Escorial revised criteria [3] with clear clinical upper and lower motor neuron signs, no more than 24 months of disease duration. A small sample of patients was studied because our preliminary study [9] suggests that a measurable effect could be detected even in very small samples. Twenty patients (10 men and 10 women; mean age years 61.2 ± 10.7 S.D.) with definite ALS [3] were enrolled and were allocated to the treatment: 10 active and 10 sham stimulation. Patients were randomly allocated by one of the authors (VD) not involved in follow-up evaluations and data analysis. Stratified block randomisation was performed such that the two treatment groups were evenly balanced for disease severity, as evaluated with the revised ALS functional rating scale (ALSFRS-R) [mean ALSFRS-R 38.3 ± 7.5 (S.D.) active and 37.93 ± 7.9 (S.D.) sham, $P > 0.05$ (unpaired *t*-test)], and duration [mean duration (months) 15.3 ± 8.2 (S.D.) active and 14.8 ± 8.9 (S.D.) sham, $P > 0.05$ (unpaired *t*-test)]. The patients and the neurologists assessing the outcomes were blinded to group assignment.

All patients were taking riluzole.

Patients were evaluated at the beginning of the treatment and every month until the end of the study at 6 months. At each visit, patients were evaluated using the ALSFRS-R and manual muscle testing (MMT). MMT testing was performed by means of the Medical Research Council (MRC) Scale. To obtain an estimate of the overall limb muscle strength we calculated a MRC compound score by adding the MRC scores of eight upper limb muscles and five lower limb muscles for each side and dividing the sum by the number of muscles tested (26 muscles). We tested the following muscles: biceps brachii, deltoid, triceps

brachii, extensor carpi radialis, extensor digitorum communis, abductor digiti minimi, abductor pollicis brevis, opponens pollicis, iliopsoas, rectus femoris, tibialis anterior, extensor hallucis longus, gastrocnemius. We also evaluated maximum voluntary isometric contraction of hand muscles (MVIC-hand) measured using a hand-held dynamometer (Biometric Ltd. Gwent, UK). For MVIC-hand, the score (in Newtons) was obtained by considering the best of three recordings obtained on each side and averaging the values obtained on the two sides.

The primary outcome measure was rate of decline of ALSFRS-R scores. Secondary outcome measures were rate of decline in MVIC-hand and in MMT. The rate of change of ALSFRS-R was chosen as primary outcome because it is easily performed and declines linearly [29].

In a subgroup of 10 patients [5 active (mean age 58.6 ± 9.4 S.D. years) and 5 sham (mean age 64 ± 7.9 S.D. years)], we evaluated the effects of a single cycle of cTBS on BDNF plasma levels. Blood samples were taken immediately before the first cTBS session and after the last cTBS session, on 5th day, of the first cycle of treatment at the same hour of the day.

BDNF was detected in sandwich ELISAs according to the manufacturer's instructions (R and D Systems, Minneapolis, MN, USA). All assays were performed on F-bottom 96-well plates (Nunc, Wiesbaden, Germany). Tertiary antibodies were conjugated to horseradish peroxidase. Wells were developed with tetramethylbenzidine and measured at 450/570 nm. Neurotrophin content was quantified against a standard curve calibrated with known amounts of protein. The detection limits was <4 pg/mL for BDNF. Measurements were performed in duplicate and are expressed as pg/ml. Cross-reactivity to related neurotrophins (NT-3; NT-4) was less than 3%.

Central motor conduction time for the first dorsal interosseous muscle was calculated by subtracting the peripheral conduction time, from spinal cord to muscles, from the latency of responses evoked by cortical stimulation at the maximum stimulator output during voluntary contraction at about 20% of maximum.

Repetitive TMS was applied over the hand motor area using a MagPro (Medtronic A/S Denmark) stimulator and a figure of eight-shaped coil.

Active rTMS was performed using the cTBS pattern in which 3 pulses of stimulation are given at 50 Hz, repeated every 200 ms for a total of 600 pulses [12]. We used a butterfly coil (MCF-B-65) with the handle pointed posteriorly and approximately perpendicular to the central sulcus. The initial direction of the current induced in the brain was posterior-anterior. The stimulation intensity was 80% of the active motor threshold (AMT), defined as the minimum single pulse intensity required to produce a motor evoked potential greater than $200 \mu\text{V}$ on more than five out of ten trials from the contracted contralateral first interosseous muscle. This protocol leads to pronounced and prolonged suppression of cortical excitability that reaches a maximum about 5–10 min after the end of the stimulation [5,12].

Sham rTMS was performed using the same stimulator connected to the placebo butterfly coil MCF-P-B-65 which has no stimulating effect on the cortex but produces similar auditory and tactile sensations as the active coil. The site of stimulation

and the number of stimuli were identical to those used for the active magnetic rTMS.

Repetitive TMS was performed bilaterally. The order of stimulation of the two hemispheres was randomized. The stimulation of the two hemispheres was performed sequentially at an interval of one minute. The motor cortex of each side was stimulated for five consecutive days every month for six consecutive months. In the patients who had absent first dorsal interosseous motor evoked potentials after stimulation of one hemisphere, the repetitive stimulation was performed at the same intensity used for the opposite hemisphere.

At the end of the treatment the patients were asked to guess whether they had undergone real or sham stimulation.

Statistical analysis was performed using a two-factor (TIME and TREATMENT) repeated measures analysis of variance. Post hoc analysis was performed using Fisher's exact PLSD test.

One active patient refused to continue the stimulation after the first day of the first cycle of stimulation because of the difficulty in reaching the Hospital, one active patient developed an unrelated medical condition (breast cancer) 2 months after the beginning of the treatment and stopped the treatment, one sham patient developed an unrelated medical condition (ileus) 2 months after the beginning of the treatment and died. One sham patient died for an ALS related cause (respiratory failure) 4 months after the beginning of treatment, one active patient was enrolled in a different trial after 4 months and was excluded from the final analysis, thus 15 patients (7 active and 8 sham) were included in the 6-months analysis. Clinical, demographic and electrophysiological characteristics of these 15 patients are summarised in Table 1. One real and one sham patient had no first dorsal interosseous muscle motor evoked potential after stimulation of one hemisphere, with preserved response on the opposite side. Thus, central motor conduction time and active motor threshold could not be evaluated in one side of these

patients. The central motor conduction time of the first dorsal interosseous muscle was within normal limits in one sham and in one real patient. Central motor conduction time was bilaterally above the normal limits of our laboratory (>7.7 ms) in two sham and in two real patients. Central motor conduction time was monolaterally prolonged or showed a pathological inter-side difference (>1 ms) in four real and in three sham patients. Active motor threshold was within normal limits in two real and in three sham patients. Active motor threshold was bilaterally above the normal limits ($>57\%$ of maximum stimulator output) in three real and in two sham patients, and above the normal limits on one side in one real and in two sham patients.

The stimulation was well tolerated by the patients and there were no side effects of rTMS. All the patients had the impression that they had undergone real stimulation. It should be considered that even real TBS was below motor threshold and thus the main sensation that is perceived by the patients is local. Sham and real stimulation result in similar auditory and tactile sensations, for this reason it is extremely difficult for a patient to distinguish between the two forms of stimulation.

The rate of decline of ALSFRS-R for active and sham patients is shown in Fig. 1A. Both active and sham patients deteriorated during treatment, with a significant effect of TIME [$F(5, 13) = 68.67$; $P < 0.0001$]. However, two-factor analysis of variance revealed a significant TIME \times TREATMENT interaction [$F(1, 5) = 5.16$; $P = 0.0005$]. This was because active rTMS patients showed a slower deterioration rate (Fig. 1A). Post hoc PLSD analysis showed a significant difference between active and sham patients only at the last control ($P = 0.035$).

The rate of decline of MMT for active and sham patients is shown in Fig. 1B. Both active and sham patients deteriorated during treatment, with a significant effect of TIME [$F(5, 13) = 25.9$; $P < 0.0001$]. However, two-factor analysis of variance revealed

Table 1
Baseline characteristics of the patients who completed the 6-months study

Characteristic		Active stimulation, $n = 7$	Sham stimulation, $n = 8$	Unpaired t -test P value
Age, years, mean (S.D.)		60.6 (13)	65.7 (7.2)	0.23
Men/women, number		5/2	3/5	
Disease duration, months, mean (S.D.)		13.9 (8.8)	14.5 (8.3)	0.89
Spinal onset, number		5	5	
Bulbar onset, number		2	3	
ALSFRS-R, mean (S.D.)		40.8 (5.5)	37.5 (7.3)	0.34
Manual muscle testing, mean (S.D.)		4.3 (0.7)	4.3 (0.6)	0.85
MVIC-hand, newton, mean (S.D.)		25.15 (5.7)	23.89 (5.4)	0.86
Active motor threshold, % MSO, mean (S.D.) ^a first dorsal interosseous muscle	Right	65 (14)	65 (19)	0.86
	Left	63 (13)	64 (17)	0.84
Central motor conduction time, ms, mean (S.D.) ^b first dorsal interosseous muscle	Right	8.9 (4.6)	9 (4.8)	0.98
	Left	9.9 (3.8)	8.5 (2.3)	0.45

ALSFRS: amyotrophic lateral sclerosis functional rating scale; MVIC: maximum voluntary isometric contraction; MSO: maximum stimulator output.

^a Normal value $< 57\%$ of MSO.

^b Normal value < 7.7 ms.

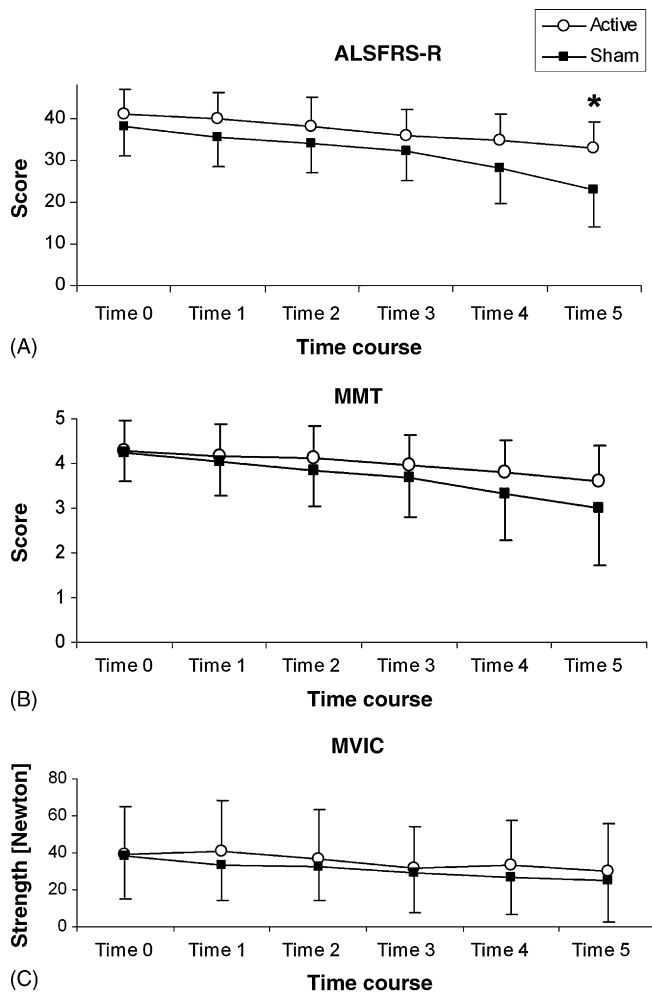


Fig. 1. (A) Rate of disease progression as evaluated with the revised ALS functional rating scale in active and sham patients. Both active and sham patients deteriorate during treatment, with a significant effect of TIME [$F(5, 13) = 68.67, p < 0.05$]. Two-factor analysis of variance reveals a significant TIME \times TREATMENT interaction [$F(1, 5) = 5.16, p < 0.05$] with a slower deterioration rate in active patients. Post hoc PLSD analysis shows a significant difference between active and sham patients only at the last control (*). (B) Rate of disease progression as evaluated with the manual muscle testing, the ordinate represents the grand mean MRC compound score obtained by adding the mean MRC scores of 26 muscles of each patient. Both active and sham patients deteriorated during treatment, with a significant effect of TIME [$F(5, 13) = 25.9, p < 0.05$]. Two-factor analysis of variance reveals a significant TIME \times TREATMENT interaction [$F(1, 5) = 2.43, p < 0.05$] with a slower deterioration rate in active patients. (C) Rate of disease progression as evaluated by measuring maximum voluntary isometric contraction of hand muscles (MVIC-hand) grand mean MRC compound score obtained by adding the mean MRC scores of 26 muscles of each patient. Both active and sham patients deteriorated during treatment, with a significant effect of TIME [$F(5, 13) = 8.76, p < 0.05$]. Two-factor analysis of variance reveals no significant difference between active and sham patients [$F(1, 5) = 0.76, p > 0.05$].

a significant TIME \times TREATMENT interaction [$F(1, 5) = 2.43; P = 0.044$]. This was because active rTMS patients showed a slower deterioration rate (Fig. 1B).

The rate of decline of MVIC-hand for active and sham patients is shown in Fig. 1C. Both active and sham patients deteriorated during treatment, with a significant effect of TIME [$F(5, 13) = 8.76, p < 0.05$]. Two-factor analysis of variance revealed

no significant difference between active and sham patients [$F(1, 5) = 0.76, p > 0.05$].

Two-factor analysis of variance revealed no significant difference in BDNF plasma levels between active and sham patients [$F(1, 1) = 0.66; p > 0.05$]. Mean BDNF plasma level in baseline conditions was 3682 ± 791 pg/ml in active and 3553 ± 223 pg/ml in sham ALS patients, after a cycle of five days of daily treatment with cTBS mean BDNF plasma level was in active 4036 ± 782 pg/ml and 3179 ± 1800 pg/ml in sham patients.

Due to the limited number of patients studied, the results of this study should be considered with caution. Our small controlled trial suggests a significant effect of cTBS of the motor cortex on the rate of decline of ALS patients as evaluated with ALSFRS-R and with MMT. The slight difference between the active and sham patients became evident after a few months and attained statistical significance only at the last control.

Toxicity to glutamate in motor neurons is mediated principally by non-NMDA receptor subtype [21]. Because the non-NMDA glutamatergic connections of the motor cortex can be activated by TMS [8] and cTBS produces long-lasting changes in motor cortex excitability, it can be hypothesised that the slight change in disease progression observed in our patients might be related to the long-lasting changes in glutamatergic neurotransmission of the motor cortex induced by cTBS. It has been demonstrated that the protocol of repetitive transcranial magnetic stimulation termed cTBS, used in the present study, determines a pronounced depression of motor cortex excitability in healthy subjects [5,12]. Thus, following cTBS, there is a decrease in the amplitude of the corticospinal volleys evoked by transcranial stimulation [5] as well as the size of the resulting motor evoked potentials [12]. In a recent study, we showed that cTBS produced a dramatic improvement in a patient with severe hemichorea-ballism, a disorder that is thought to be determined by a motor cortex hyperexcitability with benefit lasting about 24 h [6]. The suppression of motor cortex excitability in ALS patients could possibly reduce the excessive activation of glutamate receptors of corticospinal cells in ALS patients thus reducing glutamatergic excitotoxicity. If confirmed in a larger number of ALS patients, our data could suggest that the manipulation of the glutamatergic neurotransmission at the level of the motor cortex through transcranial stimulation might contrast upper motor neuron degeneration in human ALS. However, it should be considered that cTBS together with a long lasting decrease in the excitability of excitatory cortical circuits also determines a reduction in the excitability of intracortical inhibitory circuits as demonstrated by a decrease in short latency intracortical inhibition, a putative marker of GABA-A activity [12]. Because, a functional change in the activity of the intracortical inhibitory circuits has been reported in ALS patients [34], it is also possible that some of the effects of real cTBS in our patients are related to the modulations of the intracortical inhibitory circuits.

An increment in BDNF expression has been demonstrated in rat brain after long-term rTMS [15]. We evaluated the effects of a single cycle of five days of cTBS on BDNF plasma levels in a subgroup of our patients in whom the analysis of BDNF

plasma levels showed no effect of repetitive motor cortex stimulation. However, though a complete passage of intact BDNF across the blood–brain barrier by a high capacity and storable transport system, as well as its efflux from brain to blood, has been reported [16], it should be considered that BDNF plasma levels might not be correlated to the BDNF level in the brain.

Due to the extremely limited number of patients included in this pilot study, the results should be considered with caution. A further limitation of this study is represented by the slight difference in the baseline ALSFRS-R score of the two groups of patients included in the 6-months analysis. At baseline, the two treatment groups were evenly balanced for disease severity, as evaluated with ALSFRS-R, however, at 6-months analysis, because of the dropouts, the real group had a slightly higher ALSFRS-R score than the sham group. Though the difference between the two groups was not significant and it is generally assumed that the rate of decline of ALS is linear [29], we cannot rule out the possibility that the more rapid decline of the sham group was due to a more advanced stage of the disease. For these reasons, further investigations, in larger number of patients, are needed to evaluate if protocols of motor cortex stimulation that suppress cortical excitability can slow the course of the disease at least at the upper motor neuron level.

Because the effects of transcranial magnetic stimulation are short lived, cTBS is probably not suitable as a chronic intervention in ALS. However, our preliminary data support the view that the manipulation of cortical excitability through motor cortex stimulation might be useful in ALS.

In conclusion, though we cannot be sure whether the slight reduction in disease progression observed in our ALS patients can be attributed to cTBS, further studies evaluating the effects of repetitive motor cortex stimulation on disease progression in ALS patients are warranted.

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