Università degli Studi di Cagliari

PHD DEGREE

Neuroscience

Cycle XXXIII

TITLE OF THE PHD THESIS

Therapeutic use of cerebellar transcranial theta burst magnetic stimulation in movement disorders. Mechanisms of action and biomarkers of efficacy

Scientific Disciplinary Sector(s)

Area 06 – Scienze Mediche MED/26 Neurologia

PhD Student: ***Angela Maria Sanna***

Supervisor ***Prof. Monica Puligheddu***

Coordinator ***Prof Paola Fadda***

Final exam. Academic Year 2019 – 2020

Thesis defence: January 2021 Session

TABLE OF CONTENTS

Aknowledgments

I thank Prof Monica Puligheddu for giving me the chance to keep doing research in Neuroscience, for trusting and encouraging me with her fundamental advices with full respect of my passion for brain stimulation.

I am grateful to Prof. Paolo Follesa for his precious contribution in designing the research projects and performing the whole BDNF studies; I thank him particularly for his inspiring teachings on Neurobiology.

I thank Dott. Paolo Tacconi who shared with me his wide expertise in clinical neurophysiology helping me setting up brain stimulation methods and carrying out the research projects. I also thank him for always being by my side with patience and love.

I thank Mrs Daniela Boi, specialist nurse and loyal friend who helped me in the management of the patients involved in the research projects.

I thank my collegues and EEG technologists for the help and support.

Above all, I am grateful to all the patients who participated in my projects without whom all this research would have never been possible.

ABBREVIATIONS

NIBS: non-invasive brain stimulation

rTMS: repetitive transcranial magnetic stimulation

TMS: transcranial magnetic stimulation

MEPs: Motor evoked potentials

MT: motor threshold

HF: high frequency

LF: low frequency

iTBS: intermittent theta burst stimulation

cTBS: continuous theta burst stimulation

QPS: quadripulse stimulation

LTP: long term potentiation

LTD: long term depression

BDNF: Brain derived neurotrophic factor

PD: Parkinson's disease

LIDs: Levodopa-induced dyskinesias

UPDRS Unified Parkinson's Disease Rating Scale

CAPSIT: Core Assessment Program for Surgical Interventional Therapies

LEDD: Levodopa equivalent daily dose

H &Y: Hoehn and Yahr

MSA: Multiple-system atrophy

UMSARS: Unified Multiple System Atrophy Rating Scale

SCA: Spinocerebellar Ataxia

MICARS: Modified International Cooperative Ataxia Rating Scale

Summary

Movement disorders of different aetiology are characterized by an impairment in several interconnected areas of the motor system. Among the non-pharmacological options to improve motor symptoms, repetitive Transcranial Magnetic Stimulation (rTMS), represent a promising therapeutic tool due to its ability to induce long-term modulation of synaptic plasticity and its low incidence of side effects. Theta burst stimulation (TBS), a patterned protocol of rTMS, is able to induce long lasting excitatory (intermittent TBS) and inhibitory (continuous TBS) effects on cortical excitability and its very tolerable for patients due to its short duration. The high variability of response to TBS limits its use in clinical practice, thus research is focused on the characterization of predictors of response and biomarkers of efficacy. Among these, a common polymorphism of Brain Derived neurotrophic Factor (BDNF) gene, val66met, may influence the onset and progression of several neurodegenerative disorders and may alter the response to different TMS protocols, in particular TBS, but results are conflicting.

Cerebellum is considered an interesting area of stimulation for rTMS protocols in movement disorders due to its ability to influence motor learning and control through its connections with all the areas of the motor system and its role in sensory-motor integration. Indeed, it is currently used as a target for neuromodulation in movement disorders involving different pathological mechanisms.

The aim of the present study was to test the efficacy of inhibitory and excitatory cerebellar TBS in three movement disorders with different aetiology and to search possible biomarkers influencing its therapeutic effect.

In the first project a single session of cerebellar continuous TBS (cTBS) was able to reduce levodopa-induced Dyskinesia in patients affected by Parkinson's Disease (PD) and this effect

was accompanied by a decrease in serum BDNF levels. Moreover, the presence of the Val66Met polymorphism of the BDNF gene was associated with a better response.

In the second project 15 sessions of cerebellar intermittent TBS (iTBS) were able to improve motor symptoms in patients affected by Multiple system atrophy (MSA). No variations in serum BDNF levels after iTBS treatment were observed and apparently Val66Met polymorphism did not influence the clinical response.

In the third project the excitability of primary motor cortex (M1) was increased by a single session of cerebellar iTBS in patients affected by Spino-Cerebellar ataxia 38 (SCA 38), an inherited disease characterized by mutation in the EVLOV-5 gene. iTBS was then applied for 10 sessions to the cerebellum of patients leading to an improvement of motor symptoms, especially postural stability. The Val66Met polymorphism did not influence the clinical response and the changes in motor cortex excitability.

Overall, these data provide evidence for the use of cerebellar TBS in movement disorders; moreover, they suggest that BDNF Val66Met polymorphism may influence response to TBS but results vary depending on experimental model. Finally, they underline that measures of cortical excitability may provide information about the responsivity of the motor network to neuromodulation and may help to select an appropriate therapeutic protocol. Future studies will help to select other genetic, neurophysiological and imaging biomarkers leading to a better prediction and characterization of the clinical response to TBS.

1. Introduction

Movement disorders are neurological diseases characterized by an impairment in different interconnected areas of the motor system, due to genetic, degenerative, toxic and other acquired aetiology [1]. Among the non-pharmacological strategies employed to improve clinical symptoms non-invasive brain stimulation (NIBS) techniques, such as repetitive transcranial magnetic stimulation (rTMS), represent a promising therapeutic tool due to their low incidence of side effects and their ability to induce long-term modulation of synaptic plasticity [2].

1.1 Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) is a non-invasive method introduced by Baker in 1985 to stimulate motor cortex [3]. Briefly, a short high intensity current pulse is produced in a wire (magnetic coil) producing a high intensity magnetic field which penetrates the scalp and induces an electric field in the targeted cerebral cortex thus causing ions to flow in the brain which ultimately induce brief activation or inhibition of neurons [2,4] (Fig.1.1). The shape and orientation of the coil influence the pattern and direction of the induced electric field which trigger axons rather than cell bodies.

Single pulse TMS of the motor cortex has been widely used for 30 years to assess the conduction of the descending cortico-nuclear and cortico-spinal connections in primary motor cortex (M1) by recording the induced motor evoked potentials (MEPs) [2]. Paired pulse and paired associative protocols offer the possibility to study M1 excitability through facilitation and inhibition of MEPs, with diagnostic and research purposes [5]. In early 90s it has been shown that delivering many pulses of TMS in sequence, so called repetitive TMS (rTMS), it is possible to induce neuroplastic changes in the brain which outlast the stimulation period and can be applied for therapeutic intents [6,7]. rTMS may exert facilitative or inhibitory effects which have been well described in

the motor cortex, while less studied in other brain areas until the last decade when coupling rTMS with functional brain imaging and EEG has brought new acquaintance on the effects of TMS in different brain regions [8]. TMS evokes a series of descending waves of corticospinal activity which can be recorded with epidural electrodes. The earliest wave is termed the D-wave because it is caused by direct activation of the axon of corticospinal neurons in the subcortical white matter. This wave is elicited only at high intensities of stimulation. The later waves are called I-waves because they are due to synaptic activation of the same corticospinal neurons and they are numbered in order of appearance [5]. These waves are mainly prompted during rTMS protocols. Pyramidal neurons have a high threshold of activation so they are not directly stimulated by intensities normally used in rTMS protocols, usually close to the motor threshold (MT). Conversely, they undergo a synaptic activation by surrounding interneurons displaying lower thresholds [9].

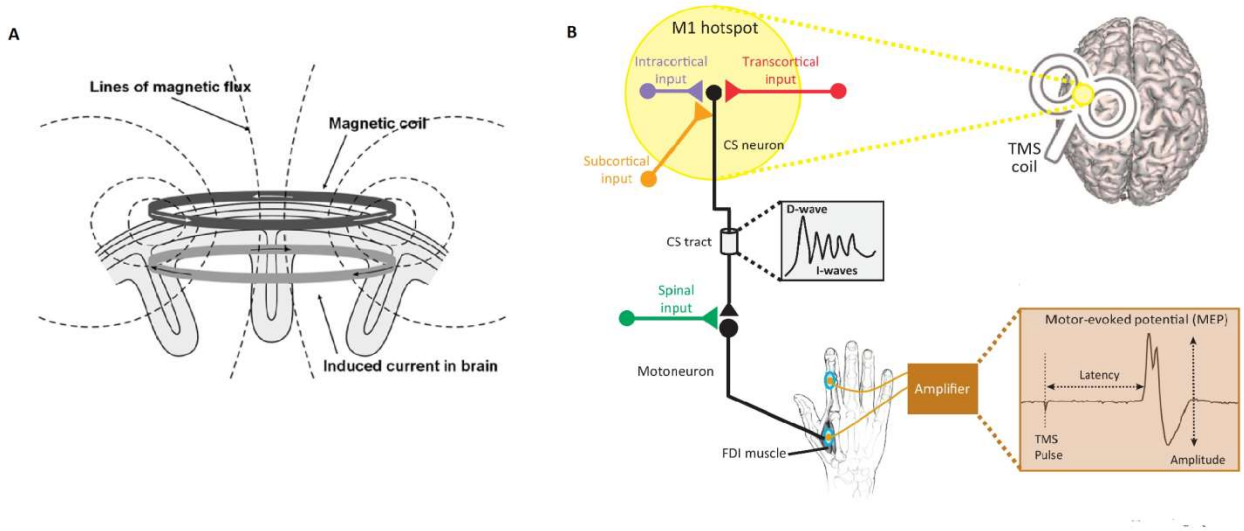


Fig. 1.1: Schematic representation of TMS action on M1. Modified from [4,10]

1.1.1 Conventional and patterned rTMS protocols. rTMS is able to excite or inhibit neuronal activity depending on the frequencies and pattern of stimulation. Classic rTMS protocols are

delivered at high frequencies (HF), with stimulus rates ≥ 5Hz, which exert an excitatory effect; low frequency protocols (LF), with stimulus rates ≤ 1Hz, produce an inhibitory effect on neuronal excitability. Patterned rTMS protocols use short rTMS burst interleaved by pauses of no stimulation such as theta burst stimulation (TBS) and quadripulse stimulation (QPS) [11] (Fig.1.2). TBS frequencies are derived from the observation of burst discharge (4–7 Hz) of the hippocampus of rats during exploratory behaviour. TMS pulses are delivered as a 3-pulse 50-Hz burst applied at 5 Hz (i.e., 50 Hz burst of 3 pulses delivered every 200 ms). Intermittent TBS (iTBS) involves 600 pulses delivered as 20 trains of 2-s of TBS followed by an 8 s rest for about 3 min and increases motor cortex excitability. Continuous TBS (cTBS) is obtained with application of one TBS train for 40 s which decreases motor cortex excitability. The produced long lasting effects on cortical excitability are based on long term potentiation and depression mechanisms (LTP and LTD) and exceed those seen with conventional rTMS protocols [12,13]. Accordingly, TBS was thought to induce effects that would have lasted more than conventional protocols but clinical studies conducted so far have shown similar efficacy over time compared to conventional protocols [14,15]. The main advantages of TBS is its short duration, making it more acceptable to participants, and the stability of stimulation parameters, which decreases the variability of parameters among different studies.

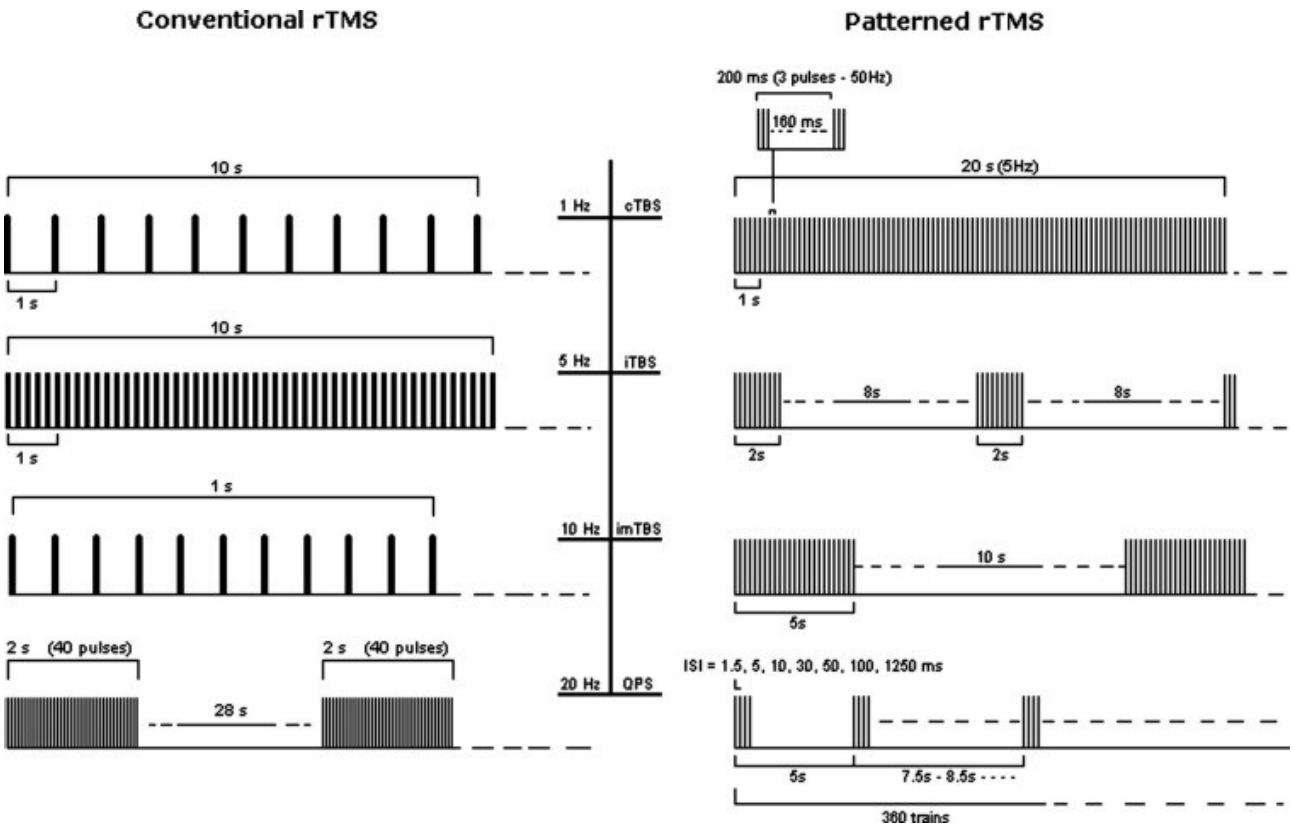


Fig. 1.2 Examples of conventional and patterned rTMS protocols [11].

1.1.2 Mechanism of action of TBS: Both excitatory and inhibitory protocols are able to induce short and long lasting changes at the primary site of activation and at secondary related areas [16–18]. The specific mechanisms of action determining therapeutic effects of rTMS and TBS protocols are still object of study and involve synaptic long term potentiation (LTP) and long term depression (LTD) mechanisms, modulation of neurotransmitter release and receptor expression, growth factors expression and activation of neuroprotection pathways [19]. It has been recently proposed a three stages theoretical model for TBS, involving mainly glutamatergic and gabaergic pathways, which initially elicits post synaptic calcium influx leading to activation of different kinases which determine whether the synapsis undergoes LTP or LTD. Afterwards synaptic long-term changes occur resulting from the sum of facilitatory and inhibitory activities in the previous stages [20]. The

secondary modulation of distant cortical and subcortical related areas by rTMS and TBS relies mostly on neurotransmitters release and secondary areas show plastic changes which may have an opposite direction of modulation from the primary target [21,22]. Thus, the resulting therapeutic effect is probably due to the modulation of large scale brain networks involving several areas controlling different physiological features [17]. Nowadays conventional and patterned protocols have been extensively applied for therapeutic purposes in several neurological and psychiatric disorders and FDA approved protocols have been released [23].

1.1.3 Variability of response. Both conventional and patterned protocols show high variability of response, depending on several factors [16,24,25]. Indeed physiological features such as age, sex, hormones, and genetic polymorphisms may influence cortical excitability and TMS response; moreover, stimulation parameters are a big source of variability. This is particularly true for conventional protocols, with many features varying among different studies thus lowering reliability, while patterned protocols such as TBS are more fixed, so less prone to be changed. Nevertheless, TBS has shown high inter- and intra-individual variability in healthy controls and in different population of patients depending on several factors such as coil orientation, genetic factors, brain state and muscle activation at the time of stimulation and others [13,26]. Consequently, technical solutions for lowering variability and better selection of individuals sensitive to different rTMS and TBS protocols have been proposed [20,24,25,27] in order to routinely employ this techniques in clinical practice.

1.2. Brain Derived Neurotrophic Factor (BDNF): influence on TMS variability. Among genetic factors modulating brain plasticity, a common polymorphism of the BDNF gene, Val66Met, which

is present in 35% of Caucasian population, is able to influence the onset and progression of several neurodegenerative disorders [28,29]. Moreover, BDNF Val66Met polymorphism has been described as an important factor contributing to the variability of response to different TMS protocols, in particular TBS [13,26]. Indeed, the polymorphism is able to alter the balance between mature BDNF and pro-BDNF, thus altering LTP and LTD mechanisms of synaptic plasticity [30].

In fact, mature BDNF is able to induce LTP trough the binding to its specific TRK-B receptor, while the interaction between pro-BDNF and p75 receptor promotes LTD. These effects involve a modulation of calcium entry in the post-synaptic membrane through the activation of specific kinases and an interaction with NMDA, AMPA and GABA receptors [30,31]. The altered physiological balance between the two forms of BDNF induced by the Val66Met polymorphism has been associated with the onset and progression of several neurodegenerative disorders with conflicting results [32]; moreover the altered synaptic plasticity associated with the presence of the polymorphism may induces changes in cortical excitability [33] and a lack of response to TMS treatment and especially to TBS protocols [13,34] which are highly dependent by LTP and LTD mechanisms and whose action is mediated by glutamatergic transmission.

1.3. Cerebellar stimulation in movement disorders: In the last years the cerebellum has gained interest as a target for neuromodulation in movement disorders [35]. In fact it plays a crucial role in motor learning and control due to its ability to modulate sensory-motor integration; moreover, it has direct and indirect connections with all cortical and sub-cortical areas of the motor system so is able to modulate different neuronal networks [36]. In physiological conditions Purkinje cells (PC) receive external sensory information and represent the only output from cerebellar cortex, targeting cerebellar deep nuclei with inhibitory synapses; dentate nucleus exerts a tonic facilitatory influence on motor areas and other regions of the neo-cortex through the interposition

of the thalamus, modulating motor and cognitive functions [36]. Thus it has been shown that targeting cerebellar cortex with single pulse TMS or inhibitory and excitatory protocols of rTMS such as iTBS and cTBS leads to short and long term changes in motor cortex excitability which may be exploited for therapeutic purposes [37]; indeed, cerebellar TMS is currently used for diagnostic and therapeutic purposes in several movement disorders with different aetiology [38–42].

Overall, among rTMS protocols TBS represent a promising therapeutic tool for movement disorders, especially due to the fact that lower intensities and shorter stimulation times make this paradigm more tolerable for patients suffering from these disabling diseases; Unfortunately the high variability of response and heterogeneity of results lower the level of evidence for its use in routinely clinical practice. Thus, ongoing research is focused in the search of predictors of response and markers of efficacy in order to select accurate conditions and appropriate population of patients suitable for treatment.

Based on these observations, the aim of the present study is to test the efficacy of TBS in three movement disorders with different aetiology and to search possible biomarkers influencing its therapeutic effect.

2. Project 1: Cerebellar continuous theta burst stimulation (cTBS) in levodopa-induced dyskinesias: role of BDNF Val66Met polymorphism and effect on serum BDNF levels.

2.1 Rationale: Patients with Parkinson Disease (PD) on chronic Levodopa therapy are likely to experience motor complications such as levodopa-induced dyskinesias (LIDs). The risk of developing LIDs has been found to be around 40% after 4-6 years of levodopa treatment and up to 94% after 15 years [43,44]. Among the genetic risk factors BDNF Val66Met polymorphysm has been reported to be associated with an early onset of LIDs [45] The complex pathophysiology of LIDs, which involves pre- and post-synaptic modifications [46,47], is not yet completely understood but several clinical and preclinical evidence consider LIDs the clinical manifestation of an aberrant plasticity involving cortical regions, basal ganglia and cerebellum [48,49]. It has been proposed that an altered sensory-motor plasticity in the striato-thalamo-cortical circuits may lead to a compensatory increase in cerebellar activity with maladaptive plastic changes. The abnormal dopaminergic levels during the peak-dose and the increased cerebellar activity make motor neurons incapable to discriminate between salient and non-salient external stimuli with an inappropriate selection of motor programs and the generation of abnormal movements [48]. Neurophysiological studies confirm an altered plasticity in PD patients with LIDs with an impairment of LTP mechanisms [50,51]. Among the different brain areas targeted with TMS to improve LIDs [38,42,52] it has been shown that cTBS applied to cerebellum is able to reduce LIDs after an acute and a chronic treatment [53] with a concomitant reduction of cerebellar metabolism as revealed by PET imaging [54]. The precise mechanism of cerebellar cTBS in improving LIDs and potential factors influencing clinical response are still unclear.

The aim of the present project is to test whether a single session of cerebellar cTBS is able to affect LID severity and BDNF serum levels in PD patients. The study also explores a possible involvement of BDNF Val66Met polymorphism in influencing the response to cTBS.

2.2 Methods

Patients: Eleven patients with PD and LIDs were enrolled. Patients underwent a complete neurological examination and Unified Parkinson's Disease Rating Scale (UPDRS) evaluation [55]. Inclusion criteria were: age ≤ 80 years, diagnosis of idiopathic PD according to Brain Bank criteria [56], stable dopaminergic treatment producing the best control of symptoms for at least 1 month before and during the study, presence of bothersome (item 33 of UPDRS ≥ 2) LIDs > 25% of waking hours (item 32 of UPDRS ≥ 2). Exclusion criteria were: previous surgery for PD, inability to understand and sign the informed consent, other neurological disorders, contraindication to rTMS. All experimental procedures were approved by local Ethical Committee. All patients signed the informed consent. The study endorsed the Principles of Human Rights, as adopted by the World Medical Association (18th WMA General Assembly) in 1964 in Helsinki (Finland) and then amended by the 64th WMA General Assembly in 2013 in Fortaleza (Brazil).

Experimental procedures: A double blind, cross over, sham-control study was performed (Fig. 2.1). Experiments were performed in the morning, between 8 and 10 am. According to Core Assessment Program for Surgical Interventional Therapies (CAPSIT) [57], patients were in withdrawal of therapy and had been fasting for 12 hours. As previously described by other Authors [53], patients received 125% of their usual morning Levodopa equivalent dose as immediate-release Levodopa/Carbidopa. After Levodopa administration patients underwent to cTBS treatment, and UPDRS III and CAPSIT dyskinesia scale were administered every 15 min for 90 min

(t0, t15, t30, t45, t60, t90). LIDs were assessed individually in seven body regions (face, neck, trunk, and right and left upper and lower limbs) and were scored as follows: 0, none; 1, mild; 2, moderate; 3, severe; and 4, extreme (total score: 0-28). Sessions were video-recorded and evaluated by two independent blinded raters.

rTMS procedure: Each patient was exposed to sham and real cerebellar cTBS stimulation in two separate sessions performed at least one week apart. The order of sessions was randomized and the patients were told that they were going to be exposed to two different protocols, causing different scalp sensation. Stimulation was applied immediately after t0 over the lateral cerebellum ipsilateral to the side in which LIDs were predominant [53]. An EB Neuro STM9000 ultra-fast stimulator connected to a 90 mm air-cooled circular coil (EB Neuro SpA, Florence, Italy) was employed. The targeting sites of the cerebellum were 1 cm inferior and 3 cm left/right to the inion. Sham stimulation was performed with the coil angled at 90° to the skull decreasing the power to 40% of AMT. The cTBS consisted of three-pulse bursts at 50 Hz repeated every 200 ms (5 Hz) for a total of 600 pulses (total time 40 s). Stimulator intensity was set at 80% of the active motor threshold (AMT) of first dorsal interosseous [12], defined as the lowest intensity to evoke a MEP of at least 200 μV in 5 out of 10 trials during slight isometric contraction of the tested muscle [2].

Genotyping: Blood was collected in appropriate EDTA coated tubes and stored at −80 °C until use. DNA was extracted from blood using a commercial kit (Sigma-Aldrich, Milan, Italy) and used as template to amplify the BDNF gene DNA region containing the rs6265 polymorphism (Genebank accession number: AB038670). In order to be 100% sure of the results, two sets of polymerase chain reaction (PCR) primers, selective for two amplicons of 401 and 273-bp both containing a common PCR–RFLP region detected using NlaIII restriction enzyme, were used as previously described with slight modifications [58,59]. Briefly, DNA was amplified by running in parallel two

PCR reactions using 0.4 µM of each set of primers (forward 5'-CCTACAGTTCCACCAGGTGAGAAGAGTG-3'; reverse 5'-ATGGATCTACGACGTTTGTACAGGTACT-3' or forward 5' AAAGAAGCAAACATCCGAGGACAAG-3'; reverse 5'-GGAGAAGAGAAAGACGACCTCCTTA-3'). The reaction mix contained: 20 ng of genomic DNA in 75 mM Tris-HCl (pH 9.0), 20 mM ammonium sulfate, 0.01% Tween 20, 1.5 mM magnesium chloride, 0.4 mM dNTP, and 1 U Taq polymerase. After an initial denaturation for 5 min at 95°C, 35 cycles of denaturating at 95°C for 30 s, annealing at 55°C for 40 s, and extension at 72°C for 50 s were performed, followed by a final extension at 72°C for 5 min. Both PCR products were digested with NlaIII to detect the G → A SNP. All samples were double-checked genotyped with each set of primers producing identical results.

Serum BDNF: Blood was collected in appropriate serum collecting tubes before (T0) and at the end (T90) of each session. Samples were centrifuged at 900 x g for 10 minutes. The resulting serum supernatant was frozen at −80 °C until use for BDNF assay. The amount of BDNF in each sample was detected with an enzyme-linked immunosorbent assay (ELISA) commercial kit (Sigma-Aldrich, Milan, Italy) as previously described [60]. ELISA was performed according to the manufacturer's instruction using a 96-well plate that was pre-coated with a primary antibody against rat BDNF. Each sample was run in duplicate. Data are presented as the mean ± SEM and are expressed as pg/ml of serum BDNF.

Statistical analysis: Graph-Pad Prism 6.01 software (La Jolla, California, USA) was used for statistical analysis. Non parametric Friedman analysis of variance was used to evaluate the effect of sham and real cerebellar cTBS on LIDs and BDNF serum levels. Post -hoc comparisons were performed by Wilcoxon tests.

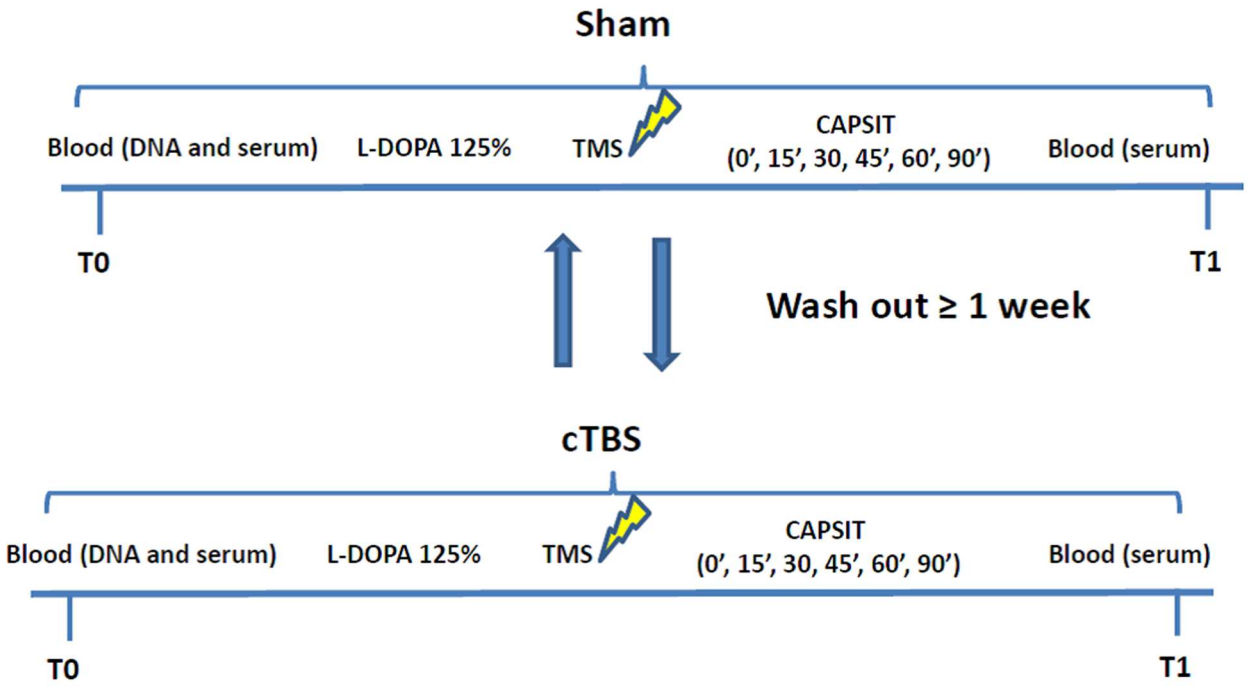


Fig. 2.1. Experimental design

2.3. Results

Participants and genotype: Table 2.1 shows the baseline characteristics, the AMT and the genotype of the patients. RFLP analysis revealed four Val66Val and seven Val66Met patients who did not differ in clinical features and in Levodopa equivalent daily dose (LEDD).

Effect of a single session of cerebellar cTBS on LIDs and influence of Val66Met polymorphism: The procedure was well tolerated by all patients and no major adverse effects were reported. Fig. 2.2A displays the effect of lateral cerebellar cTBS and sham stimulation on CAPSIT dyskinesia scores in all patients. Friedman analysis showed a significant difference between sham and real cTBS ($Q= 9.49$, $p = 0.01$). Wilcoxon post-hoc analysis showed a significant reduction in dyskinesia score at t60 for cTBS with respect to sham stimulation ($p = 0.04$). Fig. 2.2B shows the effect of cTBS on LIDs in Val66Val carriers. A significant difference was found between the two groups ($Q= 9.01$ $p = 0.04$). The reduction of CAPSIT score with cTBS compared to sham stimulation was significant at t45 ($p =$

0.03) and t60 (p = 0.03). Fig. 2.2C shows the effect of cTBS in Val66Met subjects. No significant difference between groups was found at any time point (p > 0.05)

Effect of cerebellar cTBS on serum BDNF levels: A preliminary study was conducted to investigate the effect of a single dose of Levodopa on BDNF serum levels in seven PD patients with LIDs. Levodopa alone didn't induce any significant change in serum BDNF levels (p > 0.05, data not shown). Wilcoxon test showed a significant reduction of BDNF serum levels only for real cTBS (Fig. 2.3A) (p = 0.01) but not for sham stimulation (p = 0.13). Stratifying by BDNF polymorphism, real cTBS significantly reduced serum BDNF levels in Val66Val group (Fig. 2.3B), but not in the Val66Met group (Fig. 2.3C) (p= 0.03; p 0.13, respectively).

Patient #	Age	Sex	H&Y	UPDRS	Disease duration (years)	LEDD	*BDNF* Genotype
1	55	M	3	47	11	665	Val/Val
2	72	M	2	36	14	300	Val/Met
3	74	F	3	48	7	600	Val/Val
4	62	M	3	53	15	1038	Val/Val
5	60	M	2	36	18	900	Val/Val
6	80	F	3	49	6	800	Val/Met
7	57	M	2	40	11	935	Val/Val
8	79	M	2	42	5	475	Val/Val
9	66	F	2	33	11	650	Val/Met
10	76	M	2	42	12	700	Val/Met
11	68	M	3	34	10	703	Val/Val

Table 2.1: Demographic, clinical and genetic features of patients.

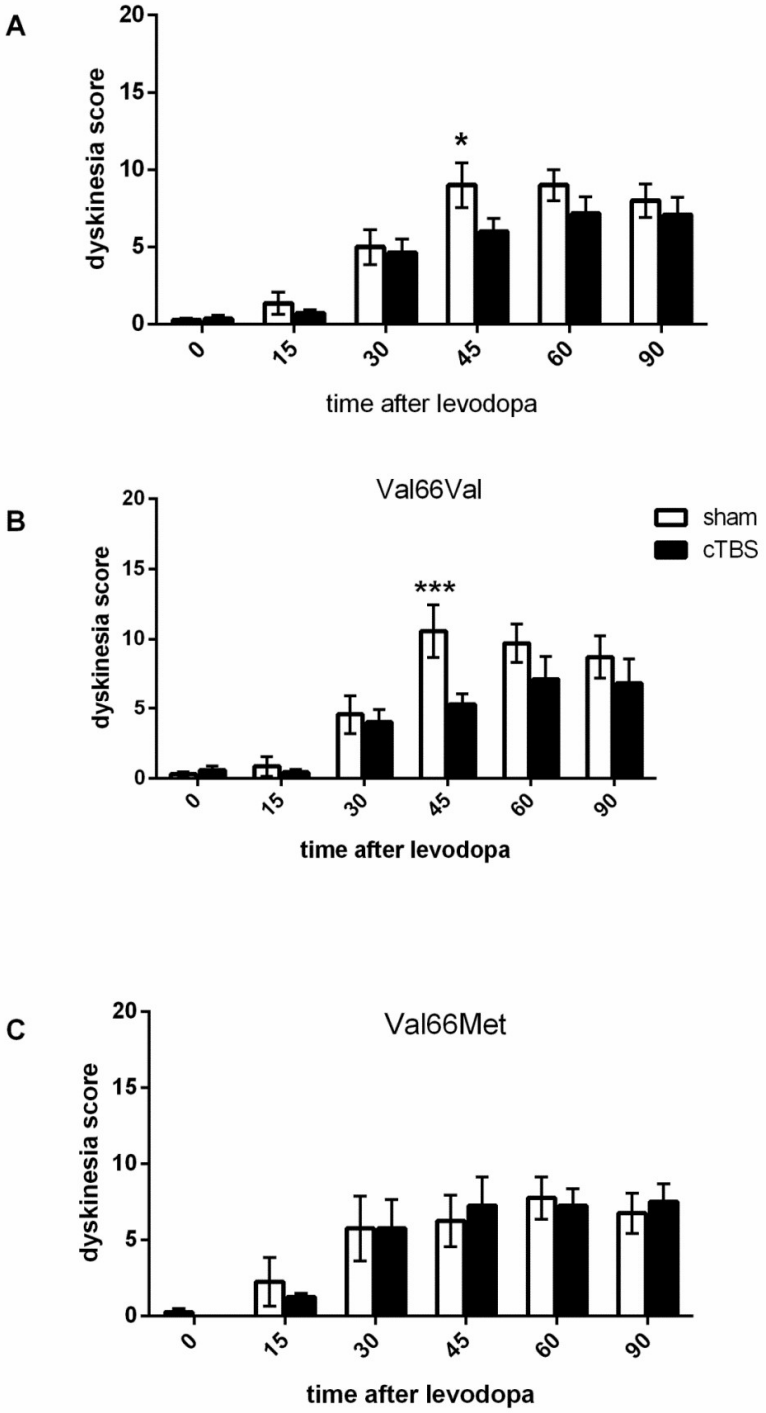


Fig.2.2: Panel A displays the effect of one session of cerebellar real and sham cTBS on dyskinesia score for all patients. Panel B shows the effect at different time points for Val66Val carriers; panel C shows the effect of cTBS at different time points for Val66Met carriers. Black and white columns indicate real and sham cTBS, respectively. Data are presented as mean ± SEM. *p < 0.05 cTBS vs sham.

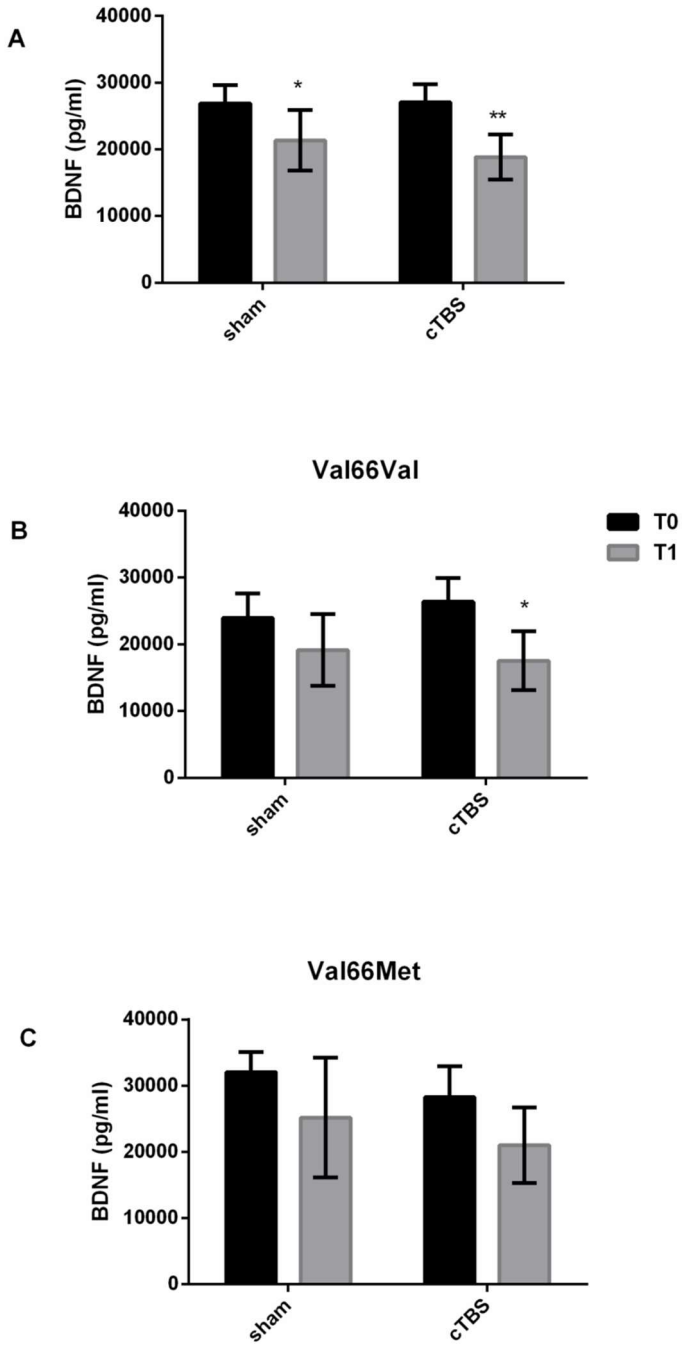


Fig. 2.3 Panel A shows the effect of one session of cerebellar real and sham cTBS on serum BDNF levels for all patients. The effect of cerebellar cTBS on serum BDNF levels in Val66Val and Val66Met patients is displayed in panel B and C, respectively. Black columns indicate T0 (before levodopa administration) and grey columns indicate T1 (90 min after levodopa administration) time points. Data are presented as mean ± SEM. *$p < 0.05$ T0 vs T1.

2.4 Discussion: This study confirms the efficacy of cerebellar cTBS in reducing LIDs [53,54] and shows that the clinical effect is accompanied by a decrease in serum BDNF levels. Moreover, Val66Met BDNF polymorphism is able to influence the clinical and biological response to cTBS.

The observed decrease of BDNF serum levels after an inhibitory TMS protocol applied to the cerebellum might be considered a LTD like mechanism which reduces motor cortex hyper-excitability as previously described in patients with LIDs [61]. The observed decrease in BDNF serum levels associated with cTBS is in agreement with a recent preclinical report on a rat model of LIDs showing that overexpression of BDNF is associated with increased susceptibility to development of LIDs, probably due to a serotoninergic hyper-innervation of basal ganglia, which in turn may exacerbate maladaptive responses to Levodopa [62]. Data on BDNF serum levels associated with rTMS are controversial, depending on the protocol and on the patients population [63,64].

This study has been published on Neurosci Lett. 2020 Jan 18;716:134653. doi: 10.1016/j.neulet.2019.134653.

3. Project 2: Effects of cerebellar intermittent theta burst stimulation (iTBS) on motor symptoms in Multiple System Atrophy (MSA)

3.1 Rationale: Multiple-system atrophy is a neurodegenerative disease characterized by progressive autonomic failure, parkinsonian features, and cerebellar and pyramidal features in various combinations [65].

The neuropathological hallmark of MSA are glial cytoplasmic inclusions consisting of misfolded α-synuclein. Selective atrophy and neuronal loss in striato-nigral and olivopontocerebellar systems underlie the division into two main motor phenotypes of MSA-parkinsonian type (MSA-P) and MSA-cerebellar type (MSA-C) A prodromal premotor phase include sexual dysfunction, urinary urge incontinence or retention, orthostatic hypotension, inspiratory stridor, and rapid-eye-movement sleep behaviour disorder which appear months to years before the first motor symptoms appear. Current pharmacological therapies target rigidity and bradykinesia, such as Levodopa, but response is inadequate and short lasting [66,67].There is no disease-modifying drug available and treatment is mostly symptomatic. There is thus an urgent need to develop new non-pharmacological therapeutic strategies to improve motor function in patients affected by MSA. Motor dysfunction in MSA is related to a cerebellar atrophy and to an altered plasticity and neuronal excitability in motor cortex and connected areas with a peculiar involvement of cerebellum, [68,69] Only few studies have explored the potential therapeutic role of rTMS in MSA, showing an improvement with conventional protocols targeting motor cortex and cerebellum, but data are still preliminary [70,71].

The aim of this study is to investigate the efficacy of iTBS applied to cerebellum, in improving clinical signs of MSA and to elucidate possible mechanisms of action.

3.2 Methods

Patients: Four patients affected by MSA-C and two patients affected by MSA-P were enrolled. Patients underwent a complete neurological examination and Unified Multiple System Atrophy Rating Scale (UMSARS) evaluation [72].

Inclusion criteria were: age ≤ 80 years, diagnosis of probable MSA with predominantly parkinsonian (MSA-P) and cerebellar (MSA-C) features according to the established consensus criteria [73]. Exclusion criteria were: Inability to understand and sign the informed consent, other severe neurological disorders, significant medical or psychiatric illnesses, history of epilepsy or seizures, pregnancy, or mental diseases. All of these patients did not respond well to the levodopa treatment. All experimental procedures were approved by local Ethical Committee. All patients signed the informed consent. The study endorsed the Principles of Human Rights, as adopted by the World Medical Association (18th WMA General Assembly) in 1964 in Helsinki (Finland) and then amended by the 64th WMA General Assembly in 2013 in Fortaleza (Brazil).

Experimental procedures: A chronic double blind cross-over treatment was performed (Fig. 3.1). Patients were randomized to receive 15 sessions of real and sham bilateral cerebellar iTBS separated by 45 days. Clinical evaluation was made by Unified Multiple System Atrophy Rating Scale (UMSARS) [72] at the beginning and at the end of each trial; patients were video-recorded and a blinded evaluator generated UMSARS scores. Blood sample were collected at the beginning and at the end of each trial.

rTMS: An EB Neuro STM9000 ultra-fast stimulator connected to a 90 mm air-cooled circular coil (EB Neuro SpA, Florence, Italy) was employed. The targeting sites of the cerebellum were 1 cm inferior and 3 cm left/right to the inion. Sham stimulation was performed with the coil angled at 90° to the skull decreasing the power to 40% of AMT. The iTBS consisted of 20 cycles of 2 sec of three-pulse bursts at 50 Hz repeated every 200 ms (5 Hz) repeated every 10 sec for a total of 600

pulses (total time 191 sec). Stimulator intensity was set at 80% of the active motor threshold (AMT) of first dorsal interosseous. Two-minute pause wase set between left/right stimulation.

Genotyping and serum BDNF analysis were performed as described in project 1.

Statistical Analysis: Graph-Pad Prism 6.01 software (La Jolla, California, USA) was used for statistical analysis. Non parametric Friedman analysis of variance was used to evaluate the effect of sham and real cerebellar iTBS on UMSARS motor score and BDNF serum levels. Post -hoc comparisons were performed by Wilcoxon tests.

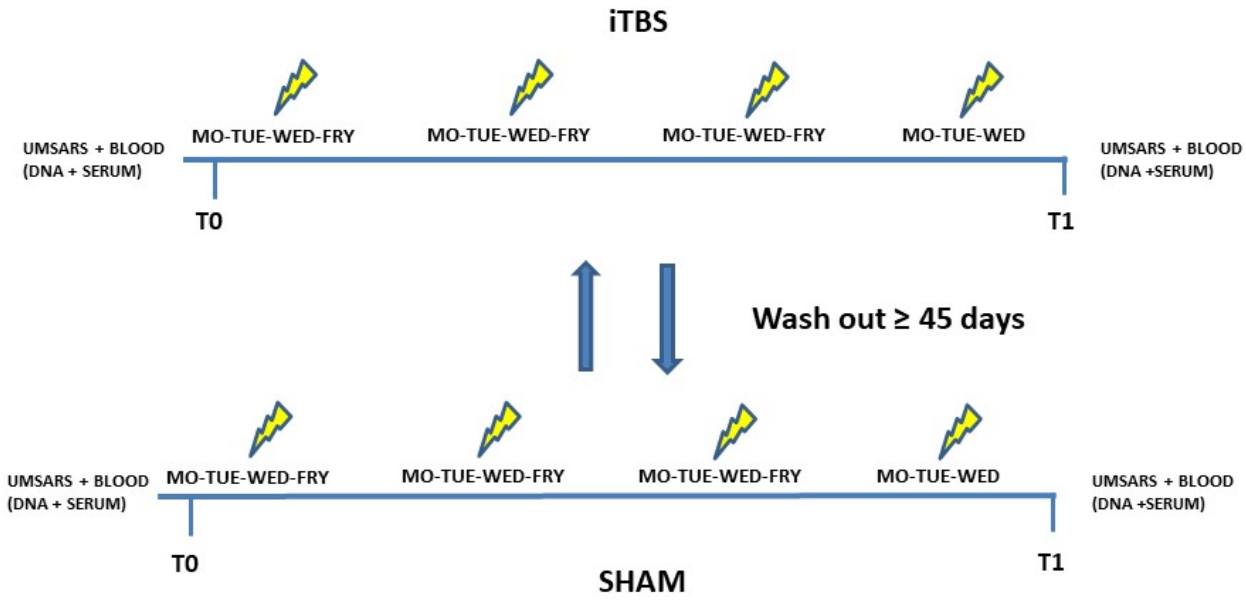


Fig 3.1: Experimental design

3.3 Results:

Patients. Table 3.1 shows the baseline characteristics, the UMSARS basal score, the AMT and the BDNF genotype of the patients. All patients completed the study without serious adverse events.

Effect of 15 sessions of cerebellar iTBS on motor score in MSA patients: Fig. 3.2 shows that cerebellar iTBS is able to induce a significant reduction of UMSARS motor score, while no effect is

observed for the sham treatment. Preliminary analysis shows that the clinical effect of iTBS was not accompanied by changes in serum BDNF ($p>0.05$) as show in Fig. 3.3

Patient #	Age	Sex	UMSARS	Disease duration (years)	*BDNF* Genotype
1	73	F	52.0	7	Val/Val
2	72	F	46.5	3	Val/Val
3	65	F	48.5	5	Val/Met
4	71	F	57.5	4	Val/Val
5	61	F	92.5	5	Val/Met
6	75	F	49.5	5	Val/Val

Table 3.2: demographic, clinical and genetic features of MSA patients.

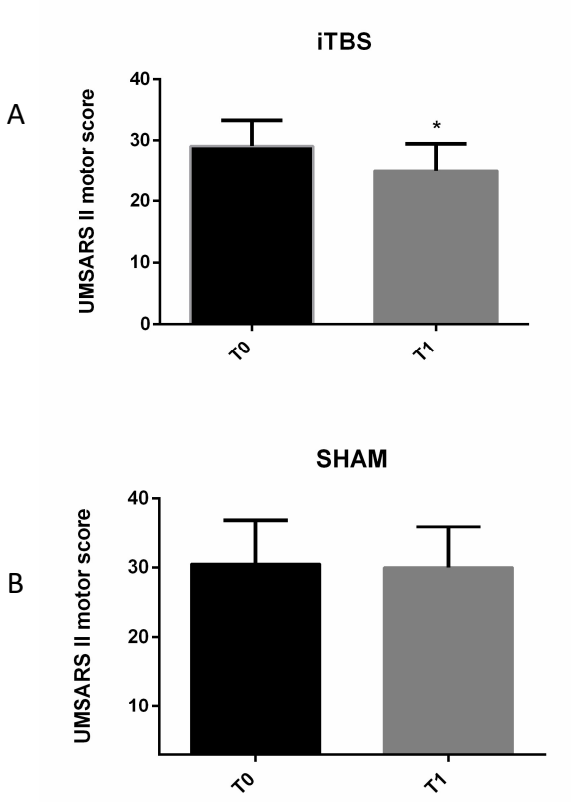


Fig. 3.2: effect of one session of cerebellar real (panel A) and sham (panel B) iTBS on UMSARS score for all patients. Data are presented as mean ± SEM. *$p < 0.05$ T1 vs T0.

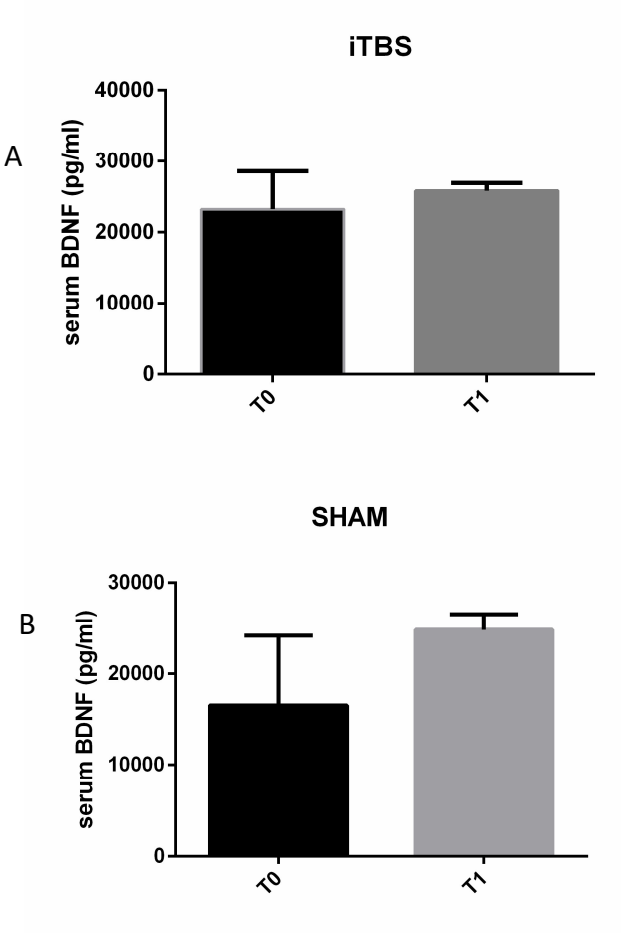


Fig. 3.3: Panel A shows the effect of one session of cerebellar real (panel A) and sham (panel B) iTBS on serum BDNF levels for MSA patients. Data are presented as mean ± SEM. *p < 0.05 T0 vs T1.

3.4 Discussion: These preliminary data show a clinical effect of bilateral cerebellar iTBS on motor score in MSA patients, confirming the potential role of cerebellar NIBS in this neurodegenerative disorder described by other authors [70,71,74]. fMRI studies have shown that HF applied to bilateral M1 is able to increase activation of the cerebellum [70]; moreover, excitatory stimulation of both cerebellum and bilateral M1 has been shown to be able to improve motor control in

patients with MSA and to increase in the physiologic complexity of the motor network. In MSA an impaired M1 excitability has been described, with a lack of response to cTBS and iTBS, probably due to an impairment in LTD/LTP plasticity [75]. This may explain the lack of modification in serum BDNF levels.

A paper with the data of the present study is in preparation.

4. Project 3: Therapeutic use of cerebellar intermittent theta burst stimulation iTBS in spinocerebellar ataxia 38 (SCA 38)

4.1 Rationale Spinocerebellar Ataxias (SCAs) are autosomal dominant neurological disorders characterized by progressive cerebellar ataxia, resulting in unsteady gait, clumsiness, and dysarthria often associated with other neurological signs such as pyramidal or extrapyramidal signs, ophthalmoplegia, and cognitive impairment. Atrophy of the cerebellum and brainstem are most often the prominent features, but other structures can be affected, leading to several phenotypes [76]. They are considered rare diseases with a prevalence ranging from 0 to 5.6 cases per 100,000 individuals. Genetically, they are grouped as repeated expansion SCAs and rare SCAs, caused by conventional mutations. The latter are generally less severe and show a slower disease progression [76,77]. The prominent clinical feature is progressive cerebellar ataxia, which usually start with unsteadiness of gait and lately involves the loss of fine motor skills; dysarthria and dysphagia are common and on clinical examination oculomotor abnormalities are present. Depending on the SCA, pyramidal and extrapyramidal signs may be present as well as sensory symptoms, epilepsy and cognitive and psychiatric disturbances [77].

SCA 38 is an autosomal dominant disorder caused by mutations in the ELOVL5 gene which encodes an enzyme involved in the synthesis of very long fatty acids, with a specific expression in cerebellar Purkinje cells [78]. Three Italian families carrying the mutation, one of which is of Sardinian descent, have been identified and characterized [79]. Brain MRI showed a selective cerebellar atrophy with normal brainstem and cerebral cortex; The disease is slowly progressive with onset at 39.1 years (range 26-50) with no sex differences. Initial clinical features are gait ataxia associated with hyposmia and pes cavus, followed by limb ataxia, dysarthria, dysphagia, ophtalmoparesis and sensory loss. By the fourth decade of the disease patients are wheel-chair bounded and dependent in daily basic activities. Mean disease duration from onset to death is 41

years. Patients do not display cognitive impairment, while anxiety disorder and hearing loss has been found in the Sardinian family [79]. Docosahexanoid acid (DHA) supplementation has been proved to be effective for improving clinical features and brain metabolism in SCA 38 at short and long term follow up [80,81], thus patients keep taking this medication daily in order to delay progression of the disease.

NIBS techniques have been proved to be effective in diagnosis and therapy of different form of ataxias [82–84] showing promising results.

The aim of this study is to investigate the efficacy of two weeks of iTBS applied to cerebellum, in improving clinical signs of SCA-38.

4.2 Methods

Patients: six patients previously diagnosed with SCA-38 [78], belonging to the same Sardinian family (Fig 4.1), were enrolled. Patients underwent a complete neurological examination and Modified International Cooperative Ataxia Rating Scale (MICARS) evaluation [85].

Inclusion criteria were: age ≤ 80 years, diagnosis of SCA 38. Exclusion criteria were: Inability to understand and sign the informed consent, other severe neurological disorders, significant medical or psychiatric illnesses, history of epilepsy or seizures, pregnancy, or mental diseases.

All experimental procedures were approved by local Ethical Committee. All patients signed the informed consent. The study endorsed the Principles of Human Rights, as adopted by the World Medical Association (18th WMA General Assembly) in 1964 in Helsinki (Finland) and then amended by the 64th WMA General Assembly in 2013 in Fortaleza (Brazil).

Experimental procedures: Changes in motor cortex excitability were evaluated after one session of cerebellar iTBS applied to 6 SCA 38. Then a chronic double blind cross over treatment (Fig 4.2) was

performed. Patients were randomized to receive 10 sessions of real and sham iTBS separated by 45 days. Clinical evaluation was made by MICARS at the beginning and at the end of each trial; patients were video-recorded and a blinded evaluator generated MICARS scores. Blood samples were collected at the beginning and at the end of each trial. Before clinical assessment nerve conduction studies were performed to evaluate the presence of polyneuropathy.

Effects of iTBS of the lateral cerebellum on MEPs: subjects were asked to relax and keep their eyes open. Single-pulse TMS was delivered through a Magstim super rapid ^{2} stimulator (The Magstim Company Ltd, Whitland, UK) connected to a figure of- eight coil placed over the left M1 in the optimal position for eliciting MEPs in the right FDI muscle. 20 single pulses delivered at the intensity able to evoke 1-mV MEPs were collected before and 10 after iTBS protocol applied to lateral right cerebellum [37,75]. iTBS was delivered at 80% of AMT as described in project 2.

rTMS procedure: Real and sham iTBS were delivered as described in project 2 every weekday for two weeks for a total of 10 sessions.

Statistical analysis: t-test was used to evaluate differences in MEP amplitude, Wilcoxon test for analysis of MICARS scale and subscales.

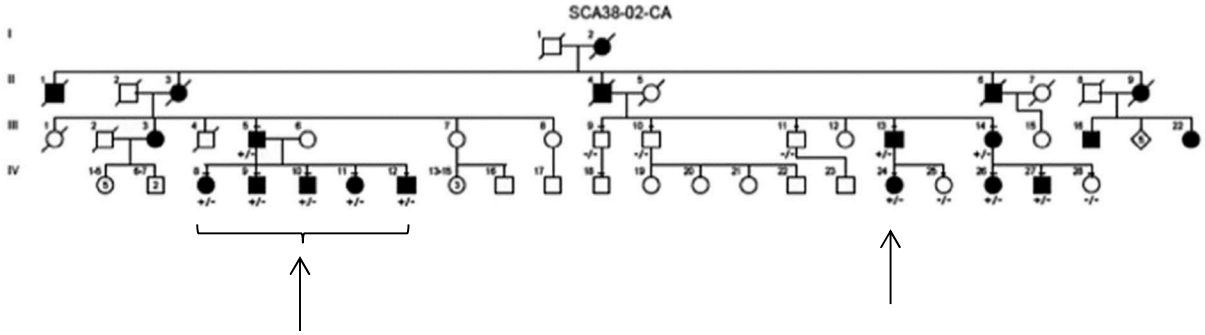
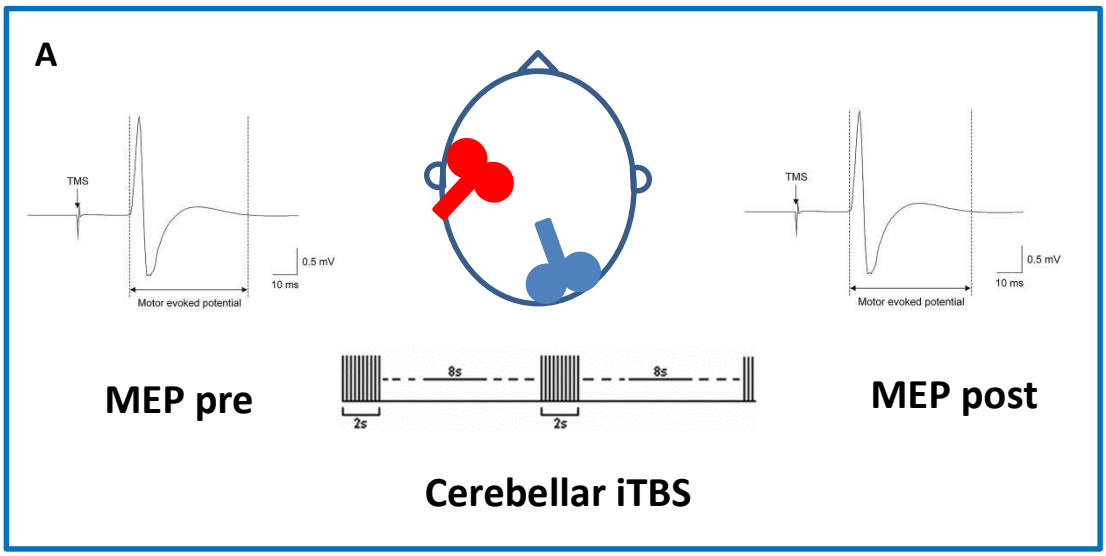


Fig. 4.1 Pedigrees of the Sardinian family with SCA38. Solid black symbols indicate affected members. Arrows indicate subjects recruited for the present study. Modified from [79]



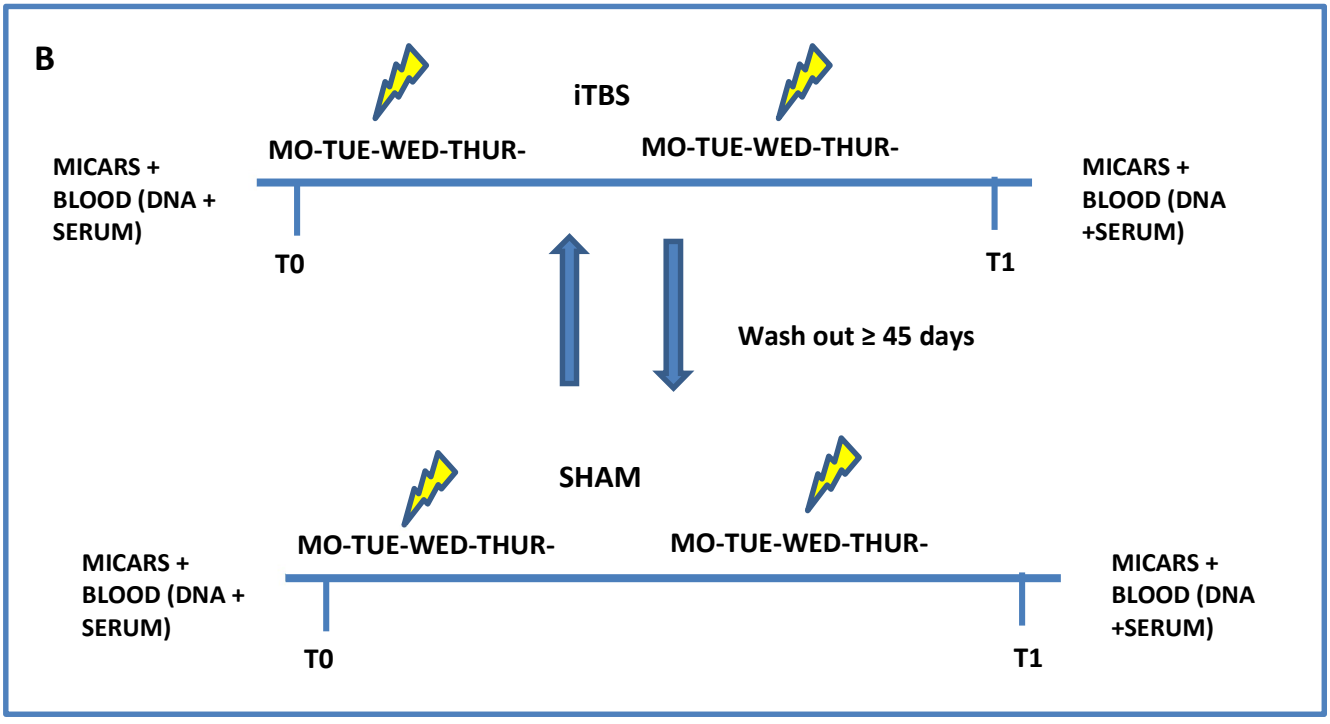


Fig. 4.2: Experimental design. Panel A shows the procedure to assess the effect of one session of cerebellar stimulation on MEP. Panel 2 shows the procedure of the chronic cerebellar iTBS treatment.

4.3 Results

Patients: table 4.1 shows demographic features, MICARS score at baseline and BDNF genotype. Nerve Conduction studies: among 6 patients only one showed signs of a sensory-motor axonal neuropathy. Other patients resulted within normal range.

Effect of a single session of cerebellar iTBS on MEPs: Fig. 4.3 shows a significant increase of MEP amplitude 10 minutes after cerebellar iTBS (1.0 ± 0.03 vs 1.7 ± 0.13; p = 0.0005). Segregating for BDNF genotype, no difference was found between Val66Val and Val66Met patients (p>0.05).

Effect of 10 sessions of cerebellar iTBS on motor symptoms: Fig. 4.4 shows a significant decrease in MICARS score after real iTBS applied to cerebellum. The total and the subscale scores were tested pre-TBS and post-TBS. Mean MICARS total scores decreased after iTBS treatment (18.83 ± 3.3 vs 14.42 ± 2.9 p=0.03). Considering the subscales, only the Posture and gait disturbance section displayed a significant decrease (Wilcoxon test p=0.02). Segregating for BDNF genotype, no difference was found between Val66Val and Val66Met patients (p>0.05).

Patient #	Age	Sex	Age at diagnosis	MICARS score	BDNF genotype
1	56	F	47	30	Val/Val
2	45	M	34	21	Val/Val
3	54	M	45	18	Val/Val
4	53	M	43	15	Val/Met
5	50	F	41	6	Val/Met
6	44	F	35	24	Val/Met

Table 4.1: Demographic, clinical and genetic features of SCA 38 patients.

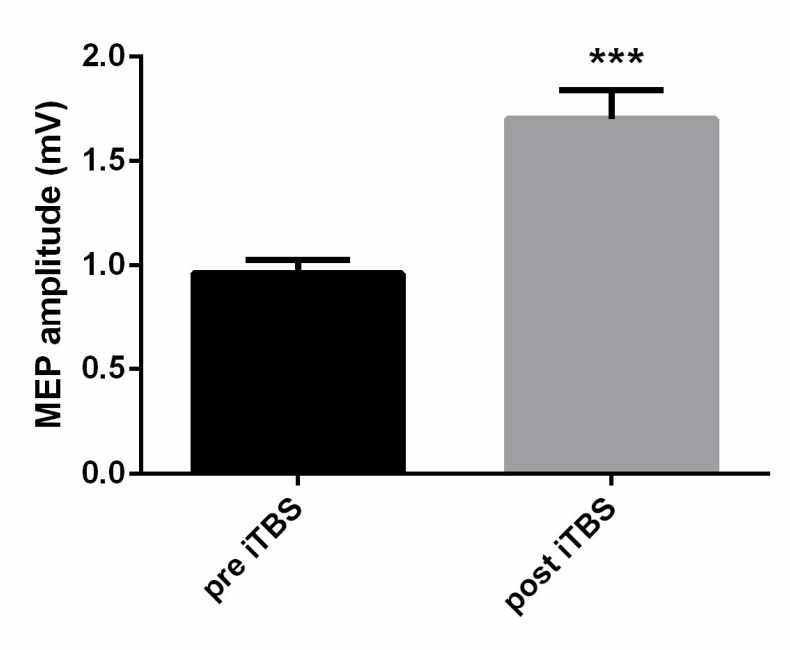


Fig. 4.3 Effect of one session of cerebellar iTBS on MEP amplitude. Data are normalized and presented as mean ± SEM. ***p < 0.001 post vs pre- iTBS.

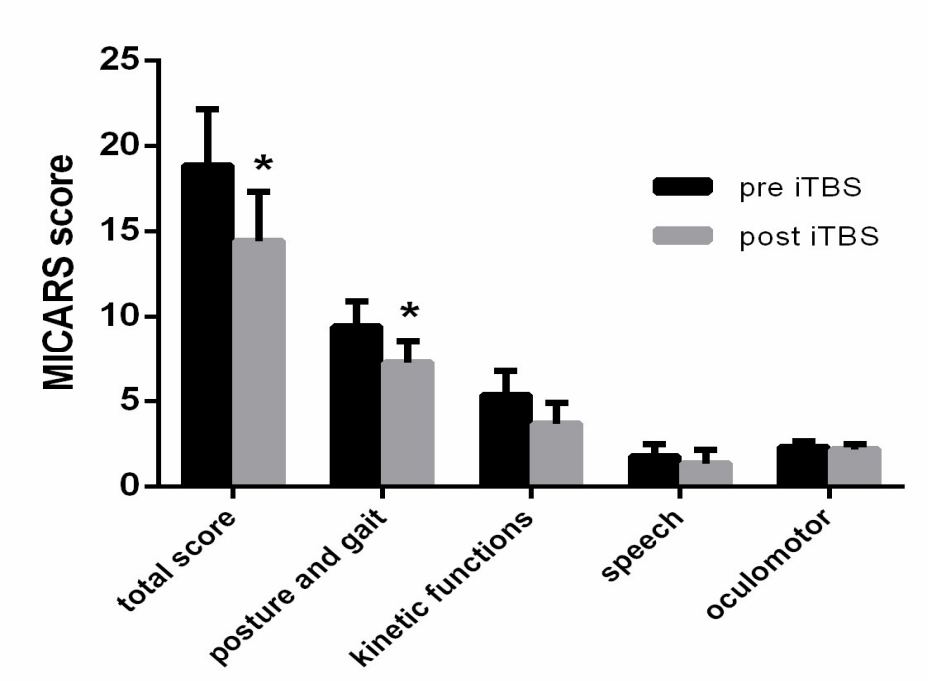


Fig. 4.4 MICARS total and subscale scores obtained by patients pre- and posttreatment. Data are presented as mean ± SEM. *p < 0.05 post vs pre- iTBS.

4.4 Discussion: this is the first repost showing a significant effect of cerebellar iTBS in improving motor symptoms in SCA-38 patients. The main effect was seen for the posture and gait section of MICARS. This data is in accord with a previous study performed on post stroke ataxic patients displaying an improvement in the same items [82]. Increasing the length of the treatment and the number of patients could eventually show a significant improvement in other clinical features which only show a non-significant tendency to improvement such as the kinetic functions and dysarthria. The observed increase of MEP in M1 after one session of cerebellar iTBS is comparable with that previously described for healthy subjects [37] and involves the cerebello-thalamus-cortical (CTC) pathway. It has been proposed that the CTC projections activated by cerebellar iTBS directly contract synapses with the GABA(B) inhibitory cortical interneurons decreasing their activity. The observed effect may be used as a predictor of response to TBS protocols in patients with neurodegenerative disorders, in which neuroplasticity is often altered, compromising response to different stimulation protocols.

A paper with the data of the present study is in preparation.

5. General discussion

The results of the present study show that excitatory and inhibitory TBS is able to improve motor symptoms in three movement disorders of different origin. In project 1 one session of cerebellar cTBS was effective in decreasing LIDs in PD patients with a concomitant reduction of serum BDNF levels. The presence of BDNF val66met polymorphism seems to impair the response to the LTD-like treatment, although the experimental sample is too small thus no conclusive assumption can be made. Nevertheless this result is in agreement with previous published data showing that the polymorphism may reduce the response to cTBS [86], although results in literature are conflicting [13,26]. The ability of cTBS to improve LIDs may be due to a decrease in the salience of external stimuli in the motor cortex which arise from an excessive cerebellar activation during L-Dopa peak [49,53].

In project 2 a chronic iTBS treatment was able to ameliorate motor symptoms in MSA patients. No changes in serum BDNF have been detected after 15 sessions of iTBS and two out of six patients are carriers of the Val66Met BDNF polymorphism. Previous studies have shown an improvement after high frequency stimulation of the cerebellum with conventional protocols either as a unique target or in combination with the motor cortex; moreover the clinical effect was associated with an increased functional connectivity in the motor system [70,71]. A clinical efficacy of cerebellar iTBS in MSA patients hasn't been described so far and needs to be confirmed with a higher number of patients. Previous studies have shown an altered cortical excitability in MSA patients in which iTBS or cTBS failed to alter MEPs when acutely administered to M1, suggesting an impairment of LTP/ LTD like plasticity. This alteration may be due to an intrinsic M1 abnormality or to an anomalous input from basal ganglia and other motor areas [75]; present data suggest that

cerebellum may be considered better target for stimulation than M1 in order to influence motor network which often display a maladaptive plasticity in degenerative disorders.

Results of project 3 show that a chronic treatment with iTBS was able to improve motor symptoms in SCA 38 patients. The analysis of MEPs, performed before and after one session of cerebellar iTBS showed that iTBS is able to increase M1 excitability in SCA 38 patients likewise healthy controls [37]. This neurophysiological marker gives information about the integrity of the cerebellar-thalamus-cortical pathway and may be used as a predictor of response before a chronic treatment with TBS or other rTMS protocols. The effect on cortical excitability was displayed by all patients and was not influenced by Val66Met BDNF polymorphism, which was found in 3 out of 6 subjects included in the study. The extent of clinical improvement after two weeks of cerebellar iTBS treatment was not apparently influenced by the polymorphism either, but a correlation analysis with a bigger sample is needed.

6. Conclusion and future directions: Overall, the data presented underline the crucial role of cerebellum as a target for movement disorders of different aetiologies either to decrease an aberrant plasticity or to improve a defective functionality of the motor network. TBS protocols due to their short duration and long-lasting effects are very manageable for operators and tolerable for patients. Unfortunately, they display an high inter and intra-individual variability of response which limit their clinical use [27]. Among predictors of response to TBS Val66Met polymorphism has shown limited value, with results varying depending on the population of patients, target area and experimental setting; other polymorphisms in genes implicated in regulating synaptic plasticity and neurodegeneration have been studied, among which polymorphisms in Catechol-O-methyltransferase (COMT), dopamine transporter (DRD2), glutamate ionotropic NMDA receptor subunits (NMDAR) have been described to play a role in influencing TMS response in physiological

and pathological conditions [16,34] but results are still controversial. Neurophysiological tests of cortical excitability have shown to be very accurate and reliable to probe the after effect of rTMS and TBS [37]; indeed they allow exploration of different neuronal pathways and an altered M1 excitability might be found even in early stages of several diseases thus leading to an early diagnosis and treatment [75,87,88]; on the other hand their execution require adequate equipment and experienced personnel thus they may be only used in a research setting. Ongoing research is combining TMS with other neurophysiological techniques such as quantitative EEG or TMS-evoked potentials in order to find better measures of efficacy and outcome parameters[20,89]. Finally, increasing the number of patients enrolled in the studies would lower the variability and increase the reliability of the observed effect, but this objective is not always achievable when treating rare and disabling diseases.

7. Bibliography

1. Fahn S: **Classification of movement disorders**. *Mov Disord* 2011, **26**.
2. Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, Di Iorio R, Di Lazzaro V, Ferreri F, Fitzgerald PB, George MS, et al.: **Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee**. *Clin Neurophysiol* 2015, **126**:1071–1107.
3. Barker AT, Jalinous R, Freeston IL: **NON-INVASIVE MAGNETIC STIMULATION OF HUMAN MOTOR CORTEX**. *Lancet* 1985, **325**:1106–1107.
4. Hallett M: **Transcranial Magnetic Stimulation: A Primer**. *Neuron* 2007, doi:10.1016/j.neuron.2007.06.026.
5. Di Lazzaro V, Rothwell JC: **Corticospinal activity evoked and modulated by non-invasive stimulation of the intact human motor cortex**. *J Physiol* 2014, **592**.
6. Pascual-leone A, Valls-solé J, Wassermann EM, Hallett M: **Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex**. *Brain* 1994, doi:10.1093/brain/117.4.847.
7. Chen R, Classen J, Gerloff C, Celnik P, Wassermann EM, Hallett M, Cohen LG: **Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation**. *Neurology* 1997, doi:10.1212/WNL.48.5.1398.
8. Burke MJ, Fried PJ, Pascual-Leone A: **Transcranial magnetic stimulation: Neurophysiological and clinical applications**. In *Handbook of Clinical Neurology*. . 2019.
9. Di Lazzaro V, Oliviero A, Pilato F, Saturno E, Dileone M, Mazzone P, Insola A, Tonali PA, Rothwell JC: **The physiological basis of transcranial motor cortex stimulation in conscious humans**. *Clin Neurophysiol* 2004, **115**.

10. Duque J, Greenhouse I, Labruna L, Ivry RB: **Physiological Markers of Motor Inhibition during Human Behavior**. *Trends Neurosci* 2017, doi:10.1016/j.tins.2017.02.006.

11. Rossi S, Hallett M, Rossini PM, Pascual-Leone A, Avanzini G, Bestmann S, Berardelli A, Brewer C, Canli T, Cantello R, et al.: **Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research**. *Clin Neurophysiol* 2009, doi:10.1016/j.clinph.2009.08.016.

12. Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC: **Theta burst stimulation of the human motor cortex**. *Neuron* 2005, doi:10.1016/j.neuron.2004.12.033.

13. Suppa A, Huang YZ, Funke K, Ridding MC, Cheeran B, Di Lazzaro V, Ziemann U, Rothwell JC: **Ten Years of Theta Burst Stimulation in Humans: Established Knowledge, Unknowns and Prospects**. *Brain Stimul* 2016, **9**.

14. Blumberger DM, Vila-Rodriguez F, Thorpe KE, Feffer K, Noda Y, Giacobbe P, Knyahnytska Y, Kennedy SH, Lam RW, Daskalakis ZJ, et al.: **Effectiveness of theta burst versus high-frequency repetitive transcranial magnetic stimulation in patients with depression (THREE-D): a randomised non-inferiority trial.** *Lancet (London, England)* 2018, **391**:1683–1692.

15. Sanna A, Fattore L, Badas P, Corona G, Cocco V, Diana M: **Intermittent Theta Burst Stimulation of the Prefrontal Cortex in Cocaine Use Disorder: A Pilot Study**. *Front Neurosci* 2019, **13**.

16. Chervyakov A V., Chernyavsky AY, Sinitsyn DO, Piradov MA: **Possible mechanisms underlying the therapeutic effects of transcranial magnetic stimulation**. *Front Hum Neurosci* 2015, **9**.

17. Cirillo G, Di Pino G, Capone F, Ranieri F, Florio L, Todisco V, Tedeschi G, Funke K, Di Lazzaro V: **Neurobiological after-effects of non-invasive brain stimulation**. *Brain Stimul* 2017, **10**.

18. Lefaucheur JP: **Transcranial magnetic stimulation**. In *Handbook of Clinical Neurology*. . 2019.

19. Huang Y-Z, Lu M-K, Antal A, Classen J, Nitsche M, Ziemann U, Ridding M, Hamada M, Ugawa Y, Jaberzadeh S, et al.: **Plasticity induced by non-invasive transcranial brain stimulation: A position paper**. *Clin Neurophysiol* 2017, **128**:2318–2329.

20. Rounis E, Huang YZ: **Theta burst stimulation in humans: a need for better understanding effects of brain stimulation in health and disease**. *Exp Brain Res* 2020, doi:10.1007/s00221-020-05880-1.

21. Strafella AP, Paus T, Fraraccio M, Dagher A: **Striatal dopamine release induced by repetitive transcranial magnetic stimulation of the human motor cortex**. *Brain* 2003, **126**:2609–2615.

22. Cho SS, Strafella AP: **rTMS of the Left Dorsolateral Prefrontal Cortex Modulates Dopamine Release in the Ipsilateral Anterior Cingulate Cortex and Orbitofrontal Cortex**. *PLoS One* 2009, **4**:e6725.

23. Lefaucheur J-P, Aleman A, Baeken C, Benninger DH, Brunelin J, Di Lazzaro V, Filipović SR, Grefkes C, Hasan A, Hummel FC, et al.: **Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS): An update (2014–2018)**. *Clin Neurophysiol* 2020, **131**:474–528.

24. Guerra A, López-Alonso V, Cheeran B, Suppa A: **Variability in non-invasive brain stimulation studies: Reasons and results**. *Neurosci Lett* 2020, **719**.

25. Guerra A, López-Alonso V, Cheeran B, Suppa A: **Solutions for managing variability in non-invasive brain stimulation studies**. *Neurosci Lett* 2020, **719**.

26. Cheeran B, Talelli P, Mori F, Koch G, Suppa A, Edwards M, Houlden H, Bhatia K, Greenwood R, Rothwell JC: **A common polymorphism in the brain-derived neurotrophic factor gene (**

BDNF) modulates human cortical plasticity and the response to rTMS. *J Physiol* 2008, doi:10.1113/jphysiol.2008.159905.

27. Corp DT, Bereznicki HGK, Clark GM, Youssef GJ, Fried PJ, Jannati A, Davies CB, Gomes-Osman J, Stamm J, Chung SW, et al.: **Large-scale analysis of interindividual variability in theta-burst stimulation data: Results from the 'Big TMS Data Collaboration.'** *Brain Stimul* 2020, doi:10.1016/j.brs.2020.07.018.

28. Zuccato C, Cattaneo E: **Brain-derived neurotrophic factor in neurodegenerative diseases**. *Nat Rev Neurol* 2009, **5**.

29. Numakawa T, Odaka H, Adachi N: **Actions of brain-derived neurotrophin factor in the neurogenesis and neuronal function, and its involvement in the pathophysiology of brain diseases**. *Int J Mol Sci* 2018, doi:10.3390/ijms19113650.

30. Zagrebelsky M, Korte M: **Form follows function: BDNF and its involvement in sculpting the function and structure of synapses**. *Neuropharmacology* 2014, **76**.

31. Mizui T, Ishikawa Y, Kumanogoh H, Kojima M: **Neurobiological actions by three distinct subtypes of brain-derived neurotrophic factor: Multi-ligand model of growth factor signaling**. *Pharmacol Res* 2016, doi:10.1016/j.phrs.2015.12.019.

32. Shen T, You Y, Joseph C, Mirzaei M, Klistorner A, Graham SL, Gupta V: **BDNF polymorphism: A review of its diagnostic and clinical relevance in neurodegenerative disorders**. *Aging Dis* 2018, doi:10.14336/AD.2017.0717.

33. Morin-Moncet O, Latulipe-Loiselle A, Therrien-Blanchet JM, Theoret H: **BDNF Val66Met polymorphism is associated with altered activity-dependent modulation of short-interval intracortical inhibition in bilateral M1**. *PLoS One* 2018, doi:10.1371/journal.pone.0197505.

34. Saghazadeh A, Esfahani SA, Rezaei N: **Genetic polymorphisms and the adequacy of brain stimulation: state of the art**. *Expert Rev Neurother* 2016,

doi:10.1080/14737175.2016.1194202.

35. França C, de Andrade DC, Teixeira MJ, Galhardoni R, Silva V, Barbosa ER, Cury RG: **Effects of cerebellar neuromodulation in movement disorders: A systematic review**. *Brain Stimul* 2018, **11**.

36. Caligiore D, Pezzulo G, Baldassarre G, Bostan AC, Strick PL, Doya K, Helmich RC, Dirkx M, Houk J, Jörntell H, et al.: **Consensus Paper: Towards a Systems-Level View of Cerebellar Function: the Interplay Between Cerebellum, Basal Ganglia, and Cortex**. *Cerebellum* 2017, **16**.

37. Koch G, Mori F, Marconi B, Codecà C, Pecchioli C, Salerno S, Torriero S, Lo Gerfo E, Mir P, Oliveri M, et al.: **Changes in intracortical circuits of the human motor cortex following theta burst stimulation of the lateral cerebellum**. *Clin Neurophysiol* 2008, doi:10.1016/j.clinph.2008.08.008.

38. Obeso I, Cerasa A, Quattrone A: **The effectiveness of transcranial brain stimulation in improving clinical signs of hyperkinetic movement disorders**. *Front Neurosci* 2016, **9**.

39. Miterko LN, Baker KB, Beckinghausen J, Bradnam L V., Cheng MY, Cooperrider J, DeLong MR, Gornati S V., Hallett M, Heck DH, et al.: **Consensus Paper: Experimental Neurostimulation of the Cerebellum**. *Cerebellum* 2019, **18**.

40. Ferrucci R, Bocci T, Cortese F, Ruggiero F, Priori A: **Noninvasive Cerebellar Stimulation as a Complement Tool to Pharmacotherapy**. *Curr Neuropharmacol* 2018, **17**.

41. Spampinato DA, Celnik PA, Rothwell JC: **Cerebellar-motor cortex connectivity: One or two different networks?** *J Neurosci* 2020, **40**.

42. Latorre A, Rocchi L, Berardelli A, Bhatia KP, Rothwell JC: **The use of transcranial magnetic stimulation as a treatment for movement disorders: A critical review**. *Mov Disord* 2019, doi:10.1002/mds.27705.

43. Tran TN, Vo TNN, Frei K, Truong DD: **Levodopa-induced dyskinesia: clinical features, incidence, and risk factors**. *J Neural Transm* 2018, doi:10.1007/s00702-018-1900-6.

44. Vijayakumar D, Jankovic J: **Drug-Induced Dyskinesia, Part 1: Treatment of Levodopa-Induced Dyskinesia**. *Drugs* 2016, doi:10.1007/s40265-016-0566-3.

45. Foltynie T, Cheeran B, Williams-Gray CH, Edwards MJ, Schneider SA, Weinberger D, Rothwell JC, Barker RA, Bhatia KP: **BDNF val66met influences time to onset of levodopa induced dyskinesia in Parkinson's disease**. *J Neurol Neurosurg Psychiatry* 2009, doi:10.1136/jnnp.2008.154294.

46. Bastide MF, Meissner WG, Picconi B, Fasano S, Fernagut PO, Feyder M, Francardo V, Alcacer C, Ding Y, Brambilla R, et al.: **Pathophysiology of L-dopa-induced motor and non-motor complications in Parkinson's disease**. *Prog Neurobiol* 2015, doi:10.1016/j.pneurobio.2015.07.002.

47. Picconi B, Hernández LF, Obeso JA, Calabresi P: **Motor complications in Parkinson's disease: Striatal molecular and electrophysiological mechanisms of dyskinesias**. *Mov Disord* 2018, doi:10.1002/mds.27261.

48. Kishore A, Popa T: **Cerebellum in levodopa-induced dyskinesias: The unusual suspect in the motor network**. *Front Neurol* 2014, **5 AUG**.

49. Rajan R, Popa T, Quartarone A, Ghilardi MF, Kishore A: **Cortical plasticity and levodopa-induced dyskinesias in Parkinson's disease: Connecting the dots in a multicomponent network**. *Clin Neurophysiol* 2017, doi:10.1016/j.clinph.2017.03.043.

50. Morgante F, Espay AJ, Gunraj C, Lang AE, Chen R: **Motor cortex plasticity in Parkinson's disease and levodopa-induced dyskinesias**. *Brain* 2006, doi:10.1093/brain/awl031.

51. Suppa A, Marsili L, Belvisi D, Conte A, Iezzi E, Modugno N, Fabbrini G, Berardelli A: **Lack of LTP-like plasticity in primary motor cortex in Parkinson's disease**. *Exp Neurol* 2011,

doi:10.1016/j.expneurol.2010.11.020.

52. Cerasa A, Obeso I, Dileone M, Quattrone A: **Transcranial Non-Invasive Brain Stimulation in Parkinson's Disease Patients with Dyskinesias. Where is the Optimal Target?** *Cerebellum* 2017, doi:10.1007/s12311-016-0768-y.

53. Koch G, Brusa L, Carrillo F, Lo Gerfo E, Torriero S, Oliveri M, Mir P, Caltagirone C, Stanzione P: **Cerebellar magnetic stimulation decreases levodopa-induced dyskinesias in Parkinson disease**. *Neurology* 2009, doi:10.1212/WNL.0b013e3181ad5387.

54. Brusa L, Ceravolo R, Kiferle L, Monteleone F, Iani C, Schillaci O, Stanzione P, Koch G: **Metabolic changes induced by theta burst stimulation of the cerebellum in dyskinetic Parkinson's disease patients**. *Park Relat Disord* 2012, doi:10.1016/j.parkreldis.2011.08.019.

55. Movement Disorder Society Task Force on Rating Scales for Parkinson's Disease: **The Unified Parkinson's Disease Rating Scale (UPDRS): status and recommendations**. *Mov Disord* 2003, doi:10.1017/CBO9780511777486.018.

56. Hughes AJ, Daniel SE, Kilford L, Lees AJ: **Accuracy of clinical diagnosis of idiopathic Parkinson's disease: A clinico-pathological study of 100 cases**. *J Neurol Neurosurg Psychiatry* 1992, doi:10.1136/jnnp.55.3.181.

57. Defer GL, Widner H, Marié RM, Rémy P, Levivier M: **Core Assessment Program for Surgical Interventional Therapies in Parkinson's disease (CAPSIT-PD)**. *Mov Disord* 1999, doi:10.1002/1531-8257(199907)14:4<572::AID-MDS1005>3.0.CO;2-C.

58. Hünnerkopf R, Strobel A, Gutknecht L, Brocke B, Lesch KP: **Interaction between BDNF Val66Met and dopamine transporter gene variation influences anxiety-related traits**. *Neuropsychopharmacology* 2007, doi:10.1038/sj.npp.1301383.

59. Sheikh HI, Hayden EP, Kryski KR, Smith HJ, Singh SM: **Genotyping the BDNF rs6265 (val66met) polymorphism by one-step amplified refractory mutation system PCR**.

Psychiatr Genet 2010, doi:10.1097/YPG.0b013e32833a2038.

60. Pisu Maria G, Boero G, Garau A, Casula C, Cisci S, Biggio F, Concas A, Follesa P, Maciocco E, Porcu P, et al.: **Are preconceptional stressful experiences crucial elements for the aetiology of autism spectrum disorder? Insights from an animal model**. *Neuropharmacology* 2019, doi:10.1016/j.neuropharm.2019.107686.

61. Guerra A, Suppa A, D'Onofrio V, Di Stasio F, Asci F, Fabbrini G, Berardelli A: **Abnormal cortical facilitation and L-dopa-induced dyskinesia in Parkinson's disease**. *Brain Stimul* 2019, doi:10.1016/j.brs.2019.06.012.

62. Tronci E, Napolitano F, Muñoz A, Fidalgo C, Rossi F, Björklund A, Usiello A, Carta M: **BDNF over-expression induces striatal serotonin fiber sprouting and increases the susceptibility to L-DOPA-induced dyskinesia in 6-OHDA-lesioned rats**. *Exp Neurol* 2017, doi:10.1016/j.expneurol.2017.07.017.

63. Jiang B, He D: **Repetitive transcranial magnetic stimulation (rTMS) fails to increase serum brain-derived neurotrophic factor (BDNF)**. *Neurophysiol Clin* 2019, doi:10.1016/j.neucli.2019.05.068.

64. Schaller G, Sperling W, Richter-Schmidinger T, Mühle C, Heberlein A, Maihöfner C, Kornhuber J, Lenz B: **Serial repetitive transcranial magnetic stimulation (rTMS) decreases BDNF serum levels in healthy male volunteers**. *J Neural Transm* 2014, doi:10.1007/s00702-013-1102-1.

65. Fanciulli A, Wenning GK: **Multiple-system atrophy**. *N Engl J Med* 2015, doi:10.1056/NEJMra1311488.

66. Krismer F, Wenning GK: **Multiple system atrophy: Insights into a rare and debilitating movement disorder**. *Nat Rev Neurol* 2017, doi:10.1038/nrneurol.2017.26.

67. Fanciulli A, Stankovic I, Krismer F, Seppi K, Levin J, Wenning GK: **Multiple system atrophy**.

Int Rev Neurobiol 2019, doi:10.1016/bs.irn.2019.10.004.

68. Payoux P, Brefel-Courbon C, Ory-Magne F, Regragui W, Thalamas C, Balduyck S, Durif F, Azulay JP, Tison F, Blin O, et al.: **Motor activation in multiple system atrophy and Parkinson disease: A PET study**. *Neurology* 2010, doi:10.1212/WNL.0b013e3181f4d78f.

69. A. S, L. M, F. DS, A. L, A. P, C. C, A. B: **Primary motor cortex long-term plasticity in multiple system atrophy**. *Mov Disord* 2014,

70. Wang H, Li L, Wu T, Hou B, Wu S, Qiu Y, Feng F, Cui L: **Increased cerebellar activation after repetitive transcranial magnetic stimulation over the primary motor cortex in patients with multiple system atrophy**. *Ann Transl Med* 2016, doi:10.21037/atm.2016.03.24.

71. Liu Z, Ma H, Poole V, Wang X, Wang Z, Yang Y, Meng L, Manor B, Zhou J, Feng T: **Effects of Multi-Session Repetitive Transcranial Magnetic Stimulation (TMS) on Motor Control and Spontaneous Brain Activity in Multiple System Atrophy (MSA): A Pilot Study**. *Brain Stimul* 2019, doi:10.1016/j.brs.2018.12.247.

72. Wenning GK, Tison F, Seppi K, Sampiao C, Diem A, Yekhlef F, Ghorayeb I, Ory F, Galitzky M, Scaravilli T, et al.: **Development and validation of the Unified Multiple System Atrophy Rating Scale (UMSARS)**. *Mov Disord* 2004, doi:10.1002/mds.20255.

73. Gilman S, Wenning GK, Low PA, Brooks DJ, Mathias CJ, Trojanowski JQ, Wood NW, Colosimo C, Dürr A, Fowler CJ, et al.: **Second consensus statement on the diagnosis of multiple system atrophy**. *Neurology* 2008, doi:10.1212/01.wnl.0000324625.00404.15.

74. Benussi A, Dell'Era V, Cotelli MS, Turla M, Casali C, Padovani A, Borroni B: **Long term clinical and neurophysiological effects of cerebellar transcranial direct current stimulation in patients with neurodegenerative ataxia**. *Brain Stimul* 2017, doi:10.1016/j.brs.2016.11.001.

75. Suppa A, Marsili L, Di Stasio F, Latorre A, Khandker Parvez A, Colosimo C, Fabbrini G, Berardelli A: **Impaired primary motor cortex LTP/LTD-like plasticity in multiple system**

atrophy. *Mov Disord* 2012,

76. Durr A: **Autosomal dominant cerebellar ataxias: Polyglutamine expansions and beyond**. *Lancet Neurol* 2010, doi:10.1016/S1474-4422(10)70183-6.

77. Klockgether T, Mariotti C, Paulson HL: **Spinocerebellar ataxia**. *Nat Rev Dis Prim* 2019, doi:10.1038/s41572-019-0074-3.

78. Di Gregorio E, Borroni B, Giorgio E, Lacerenza D, Ferrero M, Lo Buono N, Ragusa N, Mancini C, Gaussen M, Calcia A, et al.: **ELOVL5 mutations cause spinocerebellar ataxia 38**. *Am J Hum Genet* 2014, doi:10.1016/j.ajhg.2014.07.001.

79. Borroni B, Di Gregorio E, Orsi L, Vaula G, Costanzi C, Tempia F, Mitro N, Caruso D, Manes M, Pinessi L, et al.: **Clinical and neuroradiological features of spinocerebellar ataxia 38 (SCA38)**. *Park Relat Disord* 2016, doi:10.1016/j.parkreldis.2016.04.030.

80. Manes M, Alberici A, Di Gregorio E, Boccone L, Premi E, Mitro N, Pasolini MP, Pani C, Paghera B, Perani D, et al.: **Docosahexaenoic acid is a beneficial replacement treatment for spinocerebellar ataxia 38**. *Ann Neurol* 2017, doi:10.1002/ana.25059.

81. Manes M, Alberici A, Di Gregorio E, Boccone L, Premi E, Mitro N, Pasolini MP, Pani C, Paghera B, Orsi L, et al.: **Long-term efficacy of docosahexaenoic acid (DHA) for Spinocerebellar Ataxia 38 (SCA38) treatment: An open label extension study**. *Park Relat Disord* 2019, doi:10.1016/j.parkreldis.2019.02.040.

82. Bonnì S, Ponzo V, Caltagirone C, Koch G: **Cerebellar theta burst stimulation in stroke patients with ataxia**. *Funct Neurol* 2014, doi:10.11138/fneur/2014.29.1.041.

83. Benussi A, Dell'Era V, Cantoni V, Bonetta E, Grasso R, Manenti R, Cotelli M, Padovani A, Borroni B: **Cerebello-spinal tDCS in ataxia A randomized, double-blind, sham-controlled, crossover trial**. *Neurology* 2018, doi:10.1212/WNL.0000000000006210.

84. Benussi A, Pascual-Leone A, Borroni B: **Non-invasive cerebellar stimulation in**

neurodegenerative ataxia: A literature review. *Int J Mol Sci* 2020, doi:10.3390/ijms21061948.

85. Schmahmann JD, Gardner R, MacMore J, Vangel MG: **Development of a brief ataxia rating scale (BARS) based on a modified form of the ICARS**. *Mov Disord* 2009, doi:10.1002/mds.22681.

86. Jannati A, Block G, Oberman LM, Rotenberg A, Pascual-Leone A: **Interindividual variability in response to continuous theta-burst stimulation in healthy adults**. *Clin Neurophysiol* 2017, doi:10.1016/j.clinph.2017.08.023.

87. Lefaucheur JP: **Motor cortex dysfunction revealed by cortical excitability studies in Parkinson's disease: Influence of antiparkinsonian treatment and cortical stimulation**. *Clin Neurophysiol* 2005, doi:10.1016/j.clinph.2004.11.017.

88. Bologna M, Suppa A, Di Stasio F, Conte A, Fabbrini G, Berardelli A: **Neurophysiological studies on atypical parkinsonian syndromes**. *Park Relat Disord* 2017, doi:10.1016/j.parkreldis.2017.06.017.

89. Tremblay S, Rogasch NC, Premoli I, Blumberger DM, Casarotto S, Chen R, Di Lazzaro V, Farzan F, Ferrarelli F, Fitzgerald PB, et al.: **Clinical utility and prospective of TMS–EEG**. *Clin Neurophysiol* 2019, **130**:802–844.