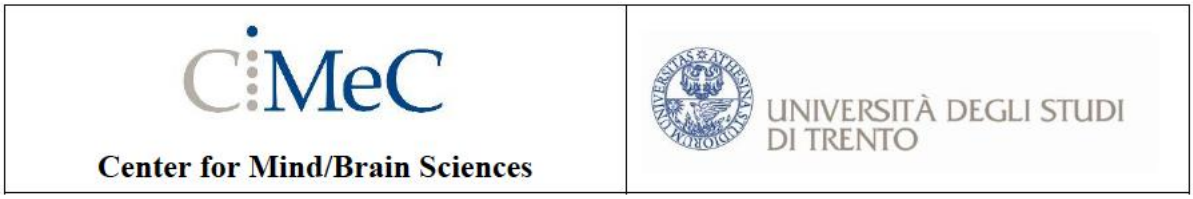

RESEARCH PROTOCOL

La stimolazione magnetica transcranica in combinazione con la riabilitazione cognitiva nel trattamento della demenza di Alzheimer

"Transcranial magnetic stimulation in combination with cognitive rehabilitation in the treatment of Alzheimer's Dementia"

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Expected duration of the research (in months): 60

STUDY RATIONALE

Brain stimulation techniques in the treatment of Alzheimer's dementia

Alzheimer's Dementia (AD) is a neurodegenerative pathology characterized by a increased progression of cognitive deficits, and respective knowledge regarding the possible mechanisms of brain reorganization and compensatory mechanisms is still scarce. Despite limited efficacy and the

presence of numerous side effects related to drug treatments, to date, cholinesterase inhibitors represent the main, if not only, treatment for patients with AD. Considering the elevated and increasing incidence of this disease, the development of alternative treatments is consequentially fundamental.

In recent years the use of non-invasive techniques of brain stimulation, and in particular, of transcranial magnetic stimulation (TMS), has obtained a heightened prominence as a potential therapeutic instrument for the treatment of diverse neurological and psychiatric pathologies (Lefaucheur et al., 2014). TMS is a noninvasive and painless method that allows the generation of an elevated (<4 Tesla) and short (<1 millisecond) magnetic pulse through a probe (solenoid - coil) placed on the head of the subject. The magnetic field passes unaltered through the extra cortical structures and induces a brief electrical current on the cortical surface below the coil, causing the depolarization of a population of cortical neurons. TMS is already widely used as a diagnostic instrument in neurological practice to the study characteristics related to the excitability of the sensorimotor cortex and the conductive properties of the corticospinal tracts (Rossini et al., 2015). When TMS is applied in a repetitive manner (rTMS), it consists of giving numerous pulses in a rapid sequence up to 200 Hz (Jung et al., 2016), and affords the possibility of investigating in an even more efficient manner both cortical activity induced modifications and neural excitability, which persist beyond the stimulation period and which have generated increasing interest in terms of its potential application as a therapeutic intervention in the cognitive rehabilitation of a vast spectrum of neuropsychiatric pathologies. (e.g., Rossi and Rossini, 2004; Lefaucheur et al., 2014).

Several studies have shown that rTMS is an effective tool for modulating brain network connectivity through the induction of changes in cortical excitability, leading to recovery or reorganization, both in local and distant, but functionally connected, regions (Bestmann et al., 2004). Therefore, the application of rTMS as a potential treatment for Alzheimer's dementia is supported by a wide variety of evidence that has identified severe connectivity alterations of the large-scale brain networks underlying AD, so much so that AD is considered a "disconnection syndrome" (Delbeuck et al., 2003; Morrison and Baxter, 2012).

Presently, there are few available studies which have evaluated the clinical impact of rTMS protocols in this population of patients. Although some studies have demonstrated an improvement in cognitive symptoms following the application of high frequency rTMS (Ahmed et al., 2012;

Bentwich et al., 2011; Cotelli et al., 2011; Giacomo Koch et al., 2017; Rabey and Dobronevsky, 2013; Rutherford et al., 2015; Lee et al., 2016; Zhao et al., 2017; Alcala-Lozano et al., 2018), there have been some conflicting results between single studies, some of which do not seem to demonstrate any beneficial effect. Indeed, the most recent line of published evidence (Lefaucheur et al., 2014) suggests that, despite encouraging results that favor the implementation of high frequency rTMS in AD in future studies, at the moment, the obtained results are not sufficient enough to justify any recommendation regarding its efficacy (Nardone et al., 2012; 2014).

"Neurorehabilitation", consisting of an integrated approach in which cognitive rehabilitation is joined in tandem with direct stimulation of the cortical areas involved in the particular function of interest which the researcher intends to rehabilitate, seems to represent the most promising approach. Therefore, TMS is used to induce the strengthening or the modulation of the altered network underlying the impaired cognitive function. The combined application of rTMS with cognitive training could represent a turning point in the assortment of interventions that aim to slow down cognitive decline resulting from AD. Numerous studies, in fact, have shown that the best way to induce the strengthening of a network is to stimulate the area while simultaneously activating the network that supports the specific function that you are aiming to improve (Barbay et al., 2006). This objective can be combined with the induction of exogenous plasticity (through the application of TMS) with specific training that itself induces exogenous plasticity (cognitive training) (Miniussi and Rossini, 2011).

New stimulation protocol: Theta Burst Stimulation (TBS)

With the scope of determining the most efficient strategy for modifying brain activity in the past few years, there have been some new protocols regarding the application of rTMS which have been developed and studied. Several studies in animal models have, in fact, demonstrated the usefulness of "bursts" of repetitive stimulation at a high theta frequency that induce synaptic plasticity. More precisely, this so-called theta-burst stimulation (TBS) consists of brief bursts of rTMS at a high frequency (50Hz) interspersed with brief pauses in which no stimulation is applied, at a frequency in the theta range (5Hz) applied continuously (cTBS), or applied intermittently (iTBS), (Huang and Kandel, 2005; Di Lazzaro et al., 2008).

Studies conducted using both types of stimulation suggest that these protocols are capable of producing long term effects on cortical excitability that exceed the effectiveness of results obtained with the use of standard rTMS protocols (Huang and Kandel, 2005; Iezzi et al., 2011). The principal advantage of TBS is that it requires a shorter period of time for administration and a lower intensity of stimulation to produce long lasting effects (Huang and Kandel, 2005). A large percentage of studies which have investigated the effect of iTBS have reported that it produces an increase in cortical excitability with the ability to persist for a considerably longer time compared with the duration of the effects gained from the use of high frequency rTMS (Di Lazzaro et al., 2011). Furthermore, the duration of TBS protocols with respect to standard protocols are much shorter. While the classic paradigms of rTMS have a duration between 20 to 45 minutes, TBS protocols require only 1 to 3 minutes of stimulation.

Recently, some studies have suggested that TBS has a greater efficacy and is better for the treatment of depression compared to that of classic rTMS, for which the anti-depressive effect is already well recognized, obtaining approval by the Food and Drug Administration as a form of treatment for drug-resistant forms of depression. In the treatment of neurological pathology, the most recent evidence of efficacy (Lefaucher et al., 2014) have demonstrated how the paradigms of cTBS show a possible treatment efficacy for neglect caused by stroke (Cazzoli et al., 2012; G. Koch et al., 2012; Nyffeler et al., 2009).

Although TBS exhibits a promising future regarding clinical interventions, further randomized, placebo-controlled studies with an adequate sample size are needed to demonstrate the true efficacy of these techniques in the scope of neurorehabilitation.

The evaluation of neuroplasticity through the co-registration of TMS-EEG

The ability of the brain to modify its functions by strengthening or inhibiting synaptic connections, to readjust its structure and/or to create new neural connections in response to environmental changes, is defined as "neuroplasticity". Neuroplasticity is the basis of learning and memory processes which occur in healthy individuals throughout the lifespan, and is of crucial importance in the scope of recovery phenomena that occur in patients following brain damage. Currently, knowledge regarding cerebral modifications related to recovery induced by treatment of neurological deficits is still limited.

Similarly, the neural mechanisms responsible for clinical improvements induced by the application of brain stimulation techniques are, for the most part, unknown. The general idea behind the use of brain stimulation is that it is able to induce changes in cortical excitability which leads to recovery and/or a reorganization of the functional networks responsible for the decline of cognitive function (Miniussi and Rossini, 2011). TMS-EEG co-registration, an innovative integrated multimodal imaging technique combines the application of single TMS pulses with electroencephalogram (EEG) recording, and its characteristics are particularly relevant to this project (Bortoletto et al., 2015). In fact, thanks to EEG recording of responses induced by TMS it is possible to test both local excitability as well as the connectivity of the cortical networks on a large scale, since activation induced by TMS in the stimulated area propagates to distant but functionally connected cortical regions.

OBJECTIVES

Primary objective:

• To evaluate the clinical, cognitive and functional efficacy of brain stimulation as a treatment, applied to patients diagnosed with mild cognitive decline (MCI) and Alzheimer's dementia (AD) in order to verify whether TBS in conjunction with cognitive rehabilitation produces better results than results obtainable through the use of only one of the two methods evaluated, based on the score of the Mini Mental State Examination or MMSE and on the scores of individual neuropsychological tests.

Secondary objectives:

• To define the most effective stimulation protocol by verifying if TBS in continuous mode (cTBS) produces better results at the behavioral level (based on scores of the neuropsychological assessment tests) than those obtainable with TBS in intermittent mode (iTBS).

• To investigate the neural mechanisms underlying neuroplasticity induced by the application of different treatment protocols, and to discover which of them is the basis for the greatest and most persistent clinical improvement. To evaluate the changes in neuroplasticity induced by the different therapeutic protocols and investigate the neural

correlates of the improvements obtained, this study will employ an innovative multimodal imaging technique: TMS-EEG co-registration.

OUTCOME MEASURES

To evaluate the efficacy of the treatment at a global level, the MMSE score will be the primary measure, while for a more in-depth evaluation of efficacy at the level of individual cognitive domains, the scores on the individual neuropsychological tests will be evaluated (primary objective and first secondary objective).

Neurophysiological indices collected through TMS-EEG co-registration will be used to evaluate any changes in neuroplasticity induced by the different therapeutic protocols and to identify a secondary marker of response to treatment. (second secondary objective).

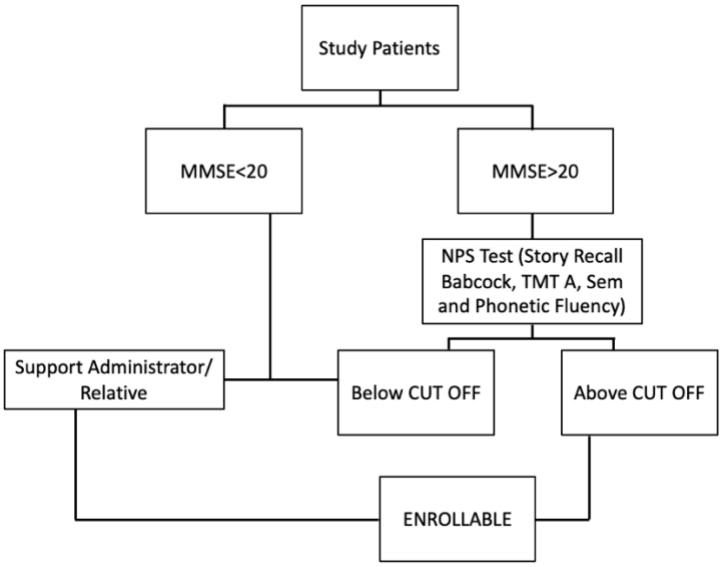
STUDY DESIGN

The project involves the enrollment of 100 patients diagnosed with Alzheimer's disease, and 100 patients diagnosed with MCI (200 participants in total) according to the criteria in the patients section.

This is a randomized, non-pharmacological study with a double-blind certified medical device (neither the patient nor the clinician/researcher who will carry out the evaluations will be aware of the group to which the patient has been assigned).

In the screening phase, the patient's ability to provide consent to participate in the research will be assessed, according to the structure shown in Figure 1. In the event that the patient does not reach normal scores on the proposed tests, formal consent to participate in the research study will be required by the support administrator.

Figure 1. Screening for the evaluation of informed consent



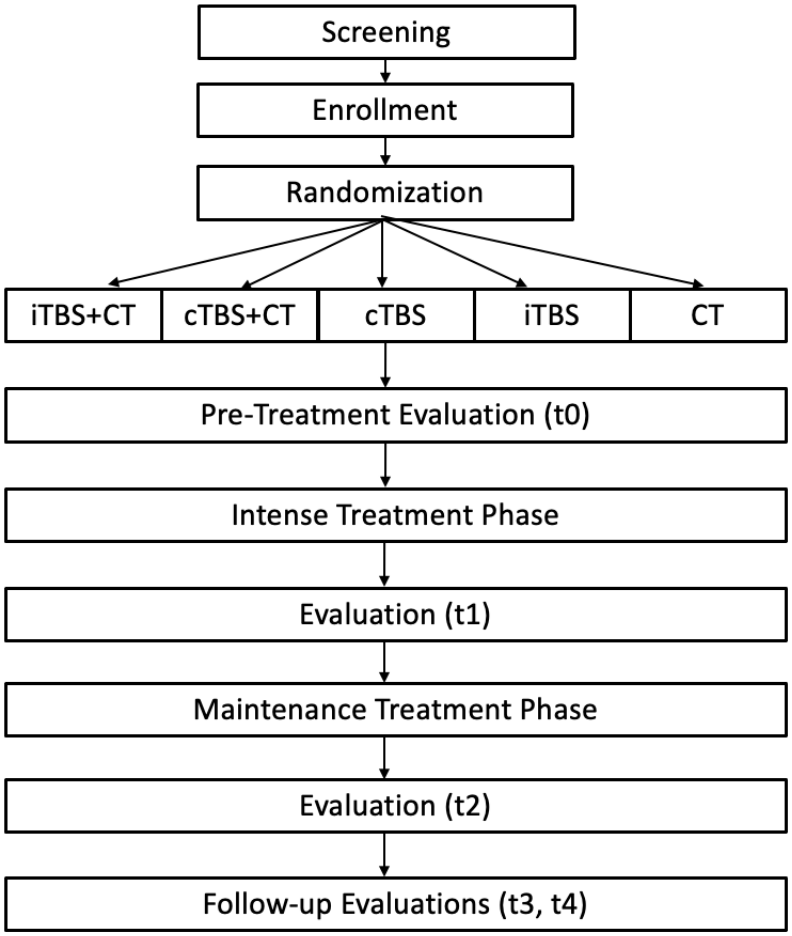
All patients included in the study will be randomly assigned to one of five different treatment protocols (20 patients diagnosed with AD and 20 patients diagnosed with MCI for each protocol), balanced using MMSE and age results so that the groups are homogeneous. In detail, the protocols are:

1. Combination of continuous TBS and cognitive training (cTBS + CT);
2. Combination of TBS in intermittent mode and cognitive training (iTBS + CT);
3. Isolated application of TBS in continuous mode (cTBS);
4. Isolated application of TBS in intermittent mode (iTBS);
5. Isolated application of cognitive training (with placebo TBS) (CT).

The treatment involves two main phases (Figure 1): 1) an intensive phase, lasting 2 weeks, with a daily frequency (5 times a week, for a total of 10 sessions); 2) a subsequent maintenance phase, lasting 5 weeks, with a biweekly frequency (2 times a week, for a total of 10 sessions).

Patients will undergo a clinical and neuropsychological evaluation and a neurophysiological evaluation before the start of treatment (baseline, t0), at the end of the intensive phase of treatment (t1), at the end of the maintenance phase (t2), and after 3 (t3) and 5 months (t4) from the start of treatment (Figure 2).

Figure 2. Study Design



Months	1				2				3				4				5				6			
Weeks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Rehabilitative Treatment																								
Combined TBS +Cognitive Training (n. sessions)		5	5	2	2	2	2	2																
Cognitive Training only (n. sessions)		5	5	2	2	2	2	2																
TBS application only (n. sessions)		5	5	2	2	2	2	2																
Evaluations																								
Clinical and Neuropsychological Evaluation	T0			T1				T2				T3								T4				
Neurophysiological Evaluation	T0			T1				T2				T3								T4				

Intensive Treatment Phase
Maintenance Phase

PATIENTS

The study will involve patients diagnosed with mild cognitive impairment (MCI) and patients diagnosed with mild to moderate Alzheimer's dementia (AD), male or female, right-handed, aged between 50 and 85 years (extreme inclusive) when the informed consent is signed, with normal or corrected vision through lenses. All patients must meet the inclusion criteria for TMS (see attached protocol). Patients will be selected on the basis of a clinical evaluation (neuropsychological test battery at the Neurocognitive Rehabilitation Center-CeRiN and, in accordance with the APSS, CSF and PET examination performed through nuclear medicine) and a further neuropsychological evaluation related to the research (see paragraph Neuropsychological evaluation).

Inclusion criteria for patients diagnosed with Alzheimer's dementia:

- Diagnosis of Alzheimer's dementia
- Mini Mental State Examination (MMSE) score ≥ 16;
- Stable intake of cholinesterase inhibitors for at least 3 months before the start of the protocol;
- Be able to provide information regarding their cognitive and functional skills, or have a caregiver available who is able to provide the patient information necessary for participation in the study and who is present when signing the patient's informed consent.

Inclusion criteria for patients diagnosed with mild cognitive impairment:

- Diagnosis of mild cognitive impairment
- Mini Mental State Examination (MMSE) score ≥ 24;
- Be able to provide information regarding their cognitive and functional skills, or have a caregiver available who is able to provide the patient information necessary for participation in the study and who is present when signing the patient's informed consent

Common exclusion criteria:

- Patients who are unable to perform the tasks required by the experimental procedure;
- History and / or evidence of any other central nervous system disorder that could be interpreted as a cause of dementia such as, for example, structural or developmental abnormality, epilepsy, infectious diseases, degenerative or inflammatory/demyelinating diseases of the central nervous system such as Parkinson's disease and Frontotemporal dementia;
- History of significant psychiatric disease which, in the investigator's judgment, could interfere with study participation;
- History of alcohol or other substance abuse, according to DSM-V criteria, or recent or previous history of drug abuse if this could be a contributing factor to dementia;
- Ongoing treatments with drugs that contain/require intake of the following substances (Rossi et al. 2009): imipramine, amitriptyline, doxepin, nortriptyline, maprotiline, chlorpromazine, clozapine, foscarnet, ganciclovir, ritonavir, amphetamines, cocaine, (MDMA, ecstasy), phencyclidine (PCP, angel dust), gamma-hydroxybutyrate acid (GHB), theophylline.
- Presence of cardiac pacemakers, electronic prostheses, biostimulators, metal inserts or electrodes implanted in the brain or skull or spine.
- Non-fulfillment of the inclusion criteria for TMS (absolute exclusion criteria) (Rossi et al., 2009), which in detail are:
    - presence of cardiac pace-makers, artificial heart valve and/or biostimulators;
    - presence of hearing aids located in the middle ear;
    - presence of metal inserts in the head and/or shoulders;

METHODS AND PROCEDURES

The materials and methods of investigation proposed will be the following:

- Administration of rTMS in theta burst mode (TBS - intermittent and continuous)
- Administration of computerized cognitive training
- Administration of a battery of neuropsychological tests
- Administration of questionnaires and scales
- Recording of the electroencephalogram (EEG)
- Combination of EEG recording with single-pulse TMS administration (TMS-EEG)

Theta burst brain stimulation protocol

rTMS will be applied using a magnetic stimulator (Magstim Super Rapid2 of which the technical data sheet, declaration of conformity and registration number in the Ministry of Health database is attached) connected to a 70 mm eight-shaped coil that generates ~ 2 Tesla maximum. Once the stimulation intensity has been defined (equal to the subjective motor threshold, i.e. at an intensity equal to 100% of the motor threshold previously determined by a surface electromyographic recording of the first dorsal interosseous of the hand), the coil will be positioned on the target area and stabilized with a mechanical support. The TBS will be applied to the left dorsolateral prefrontal cortex (DLPFC left) and the coil will be positioned at the position of the reference electrode (F3) according to the 10-20 International System.

Different TBS stimulation protocols will be applied:

1) Intermittent theta burst stimulation (iTBS): this protocol consists of the administration of 600 pulses divided into blocks of 3 pulses at 50 Hz which are applied at 5 Hz (every 200 ms), alternating 2 s of stimulation with 8 s of pause (standard iTBS protocol originally proposed by Huang and Kandel 2005).
2) Continuous theta burst stimulation (cTBS): this protocol consists of the administration of 600 pulses divided into blocks of 3 pulses at 50 Hz that are applied at 5 Hz (every 200 ms) (standard cTBS protocol originally proposed by Huang and Kandel 2005).

In both stimulation protocols, the stimulation intensity will be equal to 80% of the motor threshold value at rest. As for the protocols that involve the application of sham/placebo stimulation, the rTMS will be administered by applying a piece of wood or plastic of about 30 mm in thickness to the coil, a distance that ensures that the magnetic pulse does not reach the cortex, and will be built so that it appears to be an integral part of the apparatus (Rossi et al., 2007). All stimulation parameters adopted in this study are in accordance with the safety guidelines for the application of rTMS (Rossi et al. 2009; Rossi et al. A Consensus Statement from the IFCN Workshop on "Present and Future of TMS: Safety and Ethical Guidelines ", Siena, October 17-20, 2018).

Cognitive rehabilitation protocol

For patients assigned to the protocol including the application of cognitive training (TBS + CT; CT), the training will be administered immediately following the application of rTMS (both in the real intermittent or continuous condition, and placebo) and will last 25 minutes. Cognitive training will be administered through dedicated software that uses an individualized adaptive methodology based on the participant's performance.

The rehabilitation of memory functions, associated with the stimulation of the left DLPFC, will be focused on learning face-name associations. The face-name association training involves an acquisition phase in which patients are shown faces with an associated name and are asked to memorize these associations. The reproduction phase follows the training phase, in which the patient's task will consist of finding the face that corresponds to the associated name. Based on the patient's performance, the level of difficulty will be modulated by increasing or decreasing the number of associations to be memorized and possibly, for higher difficulty levels, by adding other information to be memorized (for example, a profession).

Neuropsychological and psychological evaluation

All patients will undergo a neuropsychological assessment before the start of treatment (t0), at the end of the intensive treatment phase (t1), at the end of the maintenance phase (t2), and after 3 (t3) and 5 months (t4) from the start of treatment (Figure 1).

The evaluation of the patients after some time (follow-up) from the end of the treatment will allow for the verification of long-term effects.

A possible “practice effect” resulting from the repeated and quick administration of neuropsychological tests is expected and will be considered in the data analysis, as in all experimental protocols of this type. The practice effect is a factor common to all experimental groups and does not affect the evaluation of the efficacy of the treatment, the primary objective of the study.

Neuropsychological assessment involves the administration of a series of standardized tests designed to investigate specific cognitive domains, such as: global cognitive functioning (*Mini Mental State Examination, MMSE*); abstract non-verbal reasoning (*Raven's Colored Progressive Matrices*); short and long term memory, both verbal and visuospatial (*Digit Span; Spatial Span; Prose Memory; Free And Cued Selective Reminding Test*; deferred re-enactment of the *Complex Figure by Rey Osterrieth*); linguistic production *(token test, semantic fluency; phonemic fluency)*; attention and executive functions (*Multiple Features Cancellation task; Trial Making test; Stroop test; attentional matrices*); and practical and visual-constructive skills (*Copy of Rey's Complex Figure*).

The psychological evaluation involves the administration of a scale that evaluates depressive symptoms in the elderly (*Geriatric Depression Scale, GDS*), a questionnaire on the quality of life and identification of deficits for the patient and for the caregiver (*QID*), a scale that evaluates awareness of deficits and disease (*Clinical Insight Rating Scale, CIRS*) and a scale to evaluate the patient's perception of empathy (*Jefferson Scale*).

Neurophysiological evaluation

Patients will undergo EEG recording and TMS-EEG co-registration before the start of treatment (t0), at the end of the intensive treatment phase (t1), at the end of the maintenance phase (t2), and after 3 (t3) and 5 months (t4) from the start of treatment (Figure 1).

The EEG will be acquired from 64 sintered Ag / AgCl electrodes placed on the scalp in accordance with the international 10-20 system, through an EEG acquisition system compatible with TMS. The EEG signal will be acquired with a high-pass filter at 0.01 Hz, a low-pass filter at 1000 Hz and with a sampling frequency of 5000 Hz. The impedance of the electrodes will be kept

below 5 kΩ. The TMS-EEG co-registration will consist of the administration of 120 pulses on the target area stimulated in the application phase of the protocol (right DLPFC or left DLPFC) at an intensity equal to 110% of the motor threshold at rest with a random frequency between 0.2- 0.4 Hz. The analysis of the data recorded by the combination of TMS-EEG will allow an in-depth evaluation of the modulations of cortical activity induced by the different treatment protocols and, in particular, will allow the investigation of cortical excitability and inhibition, cortico-cortical connectivity, and the intrinsic ability of the stimulated areas to generate oscillatory activity (Chung et al. 2015). This method will be able to provide a unique measure of local cortical activity and effective cortical-cortical connectivity (Bortoletto et al. 2015). Specifically, the analyses will focus on:

1) Potentials Evoked by TMS (TMS evoked potentials - TEP): TEPs represent the changes induced during the state of excitability/inhibition in the brain circuits following the TMS impulse. The amplitude of the TEPs will be used as a marker of cortical excitability. The analysis of TEP data will be performed using the measurements of the average amplitude values detected in successive time windows.
2) Connectivity evoked by TMS (TMS evoked connectivity): the latencies and topographical distribution of the TEPs, both at the sensor level on the scalp, and through source analysis, will represent a connectivity index and will be used to infer how the induced activity propagates from the stimulation site to functionally connected areas.
3) TMS evoked oscillations (TMS evoked oscillations): An analysis of the responses induced by TMS in the frequency domain will allow us to obtain information on the intrinsic capacity of the stimulated area to generate oscillatory activity in specific frequency bands [wavelet, synchronization and event-related desynchronization (ERS / ERD) and coherence].

The characterization and organization of brain networks will be investigated using graph theory (Stam et al. 2007).

STATISTICAL ANALYSIS

The variables that will be considered for the analysis of clinical, neuropsychological and neurophysiological data are: a) treatment effect over time (t0, t1, t2, t3, t4); b) type of treatment protocol (combination of TBS and cognitive training, isolated application of TBS, isolated application of cognitive training); c) type of stimulation protocol (cTBS, iTBS) and d) clinical group (AD or MCI). The experimental design will be both "within subjects" within each variable of interest (for example, investigating the difference between t1 and baseline to evaluate the effect of intensive treatment), and "between subjects" regarding the data between the different treatment protocols (for example, investigating the difference between combination of TBS and cognitive training and isolated application of TBS, to evaluate which protocol produces the greatest benefits), between different stimulation protocols (for example, investigating the difference between cTBS and iTBS to evaluate which protocol produces greater benefits) and between clinical conditions (to assess whether the same treatment leads to differences in the achieved benefit between the two groups of patients, AD and MCI).

Calculation of sample size

The primary outcome for the calculation of the sample size was defined as the effect of the cTBS protocol and the iTBS protocol (both in combination with cognitive training) compared to the treatment involving the combination of cognitive training with TBS placebo and the one which involves only the TBS protocol applied in isolation, on the MMSE score obtained at the end of the treatment. Based on the results of a previous rTMS study on a sample of AD patients (Ahmed et al., 2012), we estimate that at the end of our treatment there will be an improvement in the MMSE score of at least 3 points (SD of change = 2.95) for protocols that involve the combination of cognitive training and real TBS, and of 0.2 points (SD of change = 2.7) for the treatment that involves the combination of cognitive training and placebo TBS.

Considering an alpha value of 0.05 and a power of 0.80, we estimate that the number of patients to be recruited should be 16 patients per group, increased to 20 per group to take into account a possible dropout rate of 20%.

Techniques provided for data processing

Behavioral and neurophysiological data will be analyzed by analysis of variance (ANOVA) and post-hoc comparisons (t-test, contrast analysis).

Statistical processing software

Data processing will be performed with BrainVision Analyzer, SPSS and/or Statistica software.

ETHICAL CONSIDERATIONS AND ASSESSMENT OF THE RISK/BENEFIT RATIO

Expected benefits

Based on the assumptions of the present project, patients who will receive the treatment that involves the combined application of rTMS and cognitive training should show a clinical response, based on the primary endpoints reported above, better than the patients assigned to the protocols in which rTMS and cognitive training are applied in isolation. The research also provides indirect scientific/cognitive benefits, in terms of advancing knowledge on the development of treatments with proven efficacy and on the mechanisms underlying Alzheimer's dementia.

Potential Risks

The risks are represented by the use of electromedical equipment, which however all have CE authorization for use with patients. For this protocol, all appropriate safety measures will be put in place for studies with brain stimulation as indicated by the international scientific community (Rossi et al. 2009; 2018). Although, while following the international guidelines for the safe administration of TMS (Rossi et al. 2009) no adverse events are expected, it should be noted that the environment in which the research will take place and the personnel involved are able to cope with any side effects of stimulation. The stimulation parameters chosen take into account the clinical goals and safety of the participants (Rossi et al. 2009). With regard to EEG procedures, redness of the skin immediately under the electrodes is possible, following abrasion from the application of the electroconductive gel.

All the procedures foreseen by the research will be carried out paying particular attention to the patient involved, and adopting all necessary measures so that no critical issues related to stress or fatigue arise.

Risk/Benefit ratio

It is believed that in the proposed study program, the risk/benefit ratio is in favor of benefit, in terms of increased knowledge and expected direct benefit for the participants. According to the classification of the consensus paper by Rossi et al., 2009, this protocol is part of the class 2 studies which identify studies with indirect benefits and moderate risks: these are studies with patients where the clinical benefit is more speculative than anything else, but from which important data could originate for the development of effective treatments.

Ethical considerations

At the end of the study, patients will not be informed of the treatment protocol to which they have been assigned but will be informed about the overall results of the study, receiving a report containing a summary of the results achieved by the project.

Informed consent:

Participation in the study is on a voluntary basis: each subject will obtain explicit information regarding the nature of the project and will have to sign a written consent before they can be included. Participants can withdraw their consent to participate at any time, without any consequences.

Data storage and processing

The data will be protected and anonymized according to the current procedures in force. All data regarding identification will be encrypted within the database and the subjects will be identified only with a code. However, the nature of the study makes it necessary to preserve the data regarding the identification of the participants because the project provides for follow-up evaluations. Access to the database containing the collected data and the results will be restricted to the researchers involved with the project. Sensitive data and all paper data will be kept under lock and key at the various facilities. The research manager will also be responsible for the correct conservation of these data. As this study involves experimental data, the experimental data will later be published and shared with the national and international scientific community.

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