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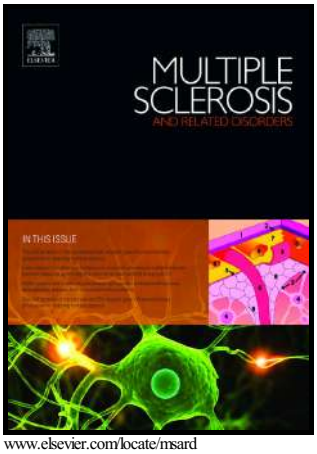

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MEG evaluation of pico-Tesla external TMS on Multiple Sclerosis patients

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Abstract

Magnetoencephalographic (MEG) recordings of 10 multiple sclerosis (MS) patients (2 men, 8 women, mean 41.3±9.5 years, mean disease duration 12.7±7.2 years) were obtained using a whole-head 122 - channel MEG system in a magnetically shielded room of low magnetic noise. Our experimental design was double-blind in order to look for possible effect of external pico - Tesla Transcranial Magnetic Stimulation (pT-TMS). The external pT-TMS was applied on the MS patients with proper field characteristics (magnetic field amplitude : 1-7.5 pT, frequency : the alpha-rhythm of the patient 8-13Hz) which were obtained prior to the application of pT-TMS. Each MS patient had two separate recording sessions consisting of 3 runs in between where were given real or sham pT-TMS. It was then tried to predict the

real and sham stimulation sessions based on the changes in the mean peak frequency difference (MPFD) observed in the brain of the patients in the 2-7 Hz frequency band. After unblinding it was found a significant effect of an increase of frequencies in the range of 2-7 Hz across the subjects followed by an improvement and normalization of the MEG.

Keywords: MEG, MS, pT-TMS, brain frequencies, double blind

Introduction

Transcranial Magnetic stimulation (TMS) is a technique with a broad variety of diagnostic and therapeutic uses in neurological conditions, including multiple sclerosis (MS) (Chen et al., 2008). It is a safe, non-invasive method and was developed as an alternative to transcranial electrical stimulation (Barker et al., 1985). Simpson and Macdonell (2015) in a review article dealing with the use of TMS in diagnosis, prognostication and treatment of MS concluded that although TMS is not widely used in clinical practice in MS, it does have the potential to supply complementary information regarding the motor system which may have uses in diagnosis, stratification, prognostication and perhaps in monitoring response to therapy. Neva et al (2016) concluded that multiple TMS-based measures of corticospinal and interhemispheric excitability provide insights into the potential neural mechanisms associated with clinical disability in MS. These findings may aid in the clinical evaluation, disease monitoring and prediction of disability in MS. Ayache et al (2016) applied TMS techniques to monitor motor cortex excitability changes in progressive MS. Meaney et al (2015) showed that consistent, repeatable TMS measures can be obtained from the resting tibialis anterior muscle of MS patients using the dual stimulators with a single pulse through a hand-held coil. Ni and Zen (2015) used TMS as potential treatment for neurodegenerative diseases (Parkinson, Alzheimer, amyotrophic lateral sclerosis, Huntington) and to understand their

pathophysiology. Houdayer et al (2015) in a review article focus on the use of electroencephalography (EEG) and TMS to study plasticity in MS. White and Petajan (2004) investigated the effects of interferon beta-1a (IFNB) therapy (Avonex) on cortically evoked motor potentials (MEPs) during resting and fatigued states in individuals with MS. They concluded that TMS utilized in this study shows promise as a physiologic measure with sensitivity to disease events and possible therapeutic effects. TMS studies in individuals with MS have shown variable sensitivities to clinical signs and symptoms (Curra et al. 2002). Schmierer et al. (2002) concluded that the combination of central motor latencies and transcallosal inhibition evoked by TMS yields objective data to estimate disease progression in MS.

In our study we used a pico Tesla TMS (pT-TMS) electronic device (Anninos and Tsagas inventors, 1995) for the therapeutic treatment of MS (**Figure 1B**). It is a modified helmet containing up to 122 coils which are arranged in five array groups, so as to cover the main 7 brain regions (frontal, vertex, right and left temporal , right and left parietal and occipital regions) of the subject. It is designed to create pT-TMS range modulations of magnetic flux in the alpha frequency range (8-13Hz) of each patient (Anninos et al., 2015, 2008, 2007a,b,2006,2003,2000, 1999,1991, 1989) (**Figure 1C**) . The pT-TMS device was configured for each individual to generate a square wave so as to resemble the firing activity of neurons in the brain (Anninos et al, 1970). The electronic device has an extra hidden switch to disable current flow to the helmet coils. This switch, controlling real or sham stimulation, was operated by a member of the technical support team, so that neither the subject nor the experimenter were aware of whether sham or real stimulation was applied (double blind design).

To our knowledge there are no other reports in the literature concerning MS, MEG and TMS. The purpose of this investigation was to examine the effects of pT-TMS on the clinical status of MS patients by means of MEG.

Methods

10 MS patients, (2 men, 8 women, mean 41.3±9.5 years, mean disease duration 12.7±7.2 years) affected by definite MS according to the criteria of Lublin and Reingold(1996)and of Neurological disability, measured using the Kurtzke (1983) expanded disability status scale (EDSS). Informed consent for the methodology and aim of the study was obtained from all participants prior to the procedure. The research was approved by the Research Committee of the Democritus University of Thrace (code number 80347). The MS were referred to our Laboratory of Medical Physics in Alexandroupoli, Greece, by practicing neurologists. The patients were off medication for 24 hours during their participation in the study.

Biomagnetic measurements were performed using a whole-head 122-channel MEG (Neuromag-122, Neuromag Ltd. Helsinki, Finland) (**Figure 1A**) .Recordings were taken in an electromagnetically shielding room in order to avoid extraneous electromagnetic noise (Anninos et al., 2015, 2008, 2007a,b, 2006, 2003, 2000, 1999,1991, 1989; Kotini et al. 2007a,b). The participants had to be free of any metal objects. In addition four indicator coils were attached to the head of each individual MS patient in order to determine the exact position of the head with respect to the MEG sensors. The exact positions of the coils were determined using a three dimensional head position indicator (HPI) digitizer.

Experimental protocol

The time taken for each recording was 2min in order to ensure alertness for each subject. Each patient was scanned in two separate sessions. During each MEG scan the subject had no task and was asked to sit comfortably in the MEG chair. The first session (**session 1**) consisted of a 2-minute resting state MEG scan. These data were subsequently used to

establish the subject's alpha frequency in the range of (8-13 Hz), for calibration of the pT-TMS electronic device (**Figure 1B**). In the second (**session 2**) scanning session, the protocol was as follows: At all times the pT-TMS electronic device which is connected to the helmet was set to real or sham stimulation by a third party. Neither the researcher nor the participant were aware of the state of the device. First, 2 minutes of pre-stimulus baseline MEG data were recorded (**run 1**). Next, 2 minutes of real or sham pT-TMS stimulation were administered with the subject sitting comfortably just outside the scanner room. Following these 2 minutes of stimulation, a further 2 minutes of resting state MEG data were acquired (**run 2**). This was followed by another 2 minutes of stimulation- in this case the device was switched from sham to real or vice versa (by the third party)- and 2 more minutes of MEG scanning data were carried out (**run 3**).

Data acquisition

The spontaneous MEG recordings were taking with sampling frequency rate at 256Hz and the associated Nyquist frequency was 128Hz ,which was well above constituent frequency components of interest in our MEG recordings and avoid aliasing artifacts. The MEG signal was filtered with cut-off frequencies at 0.3 and 40Hz. All MEG data tracings were visually inspected carefully off-line for movement artifacts and periods contaminated with movement artifacts were cut off.

Data analysis

A software program was developed in our laboratory in order to detect the amplitude of the primary dominant frequency of the power spectra of the MEG recordings obtained from each MS patient and channel after the application of Fast Fourier Transform (FFT) (**Figure 2**). Then it was interesting to look (alpha for calibration of the electronic device) and (2-7 Hz for the analysis) and as it was stated above at the primary dominant frequency of the power spectra of the MEG recordings obtained from each patient and channel after the application

of the FFT. In **Figure 2** the actual signal length for analysis is 2 min and the FFT was applied only to 8secs, and in order to explain the primary dominant frequency it was necessary to use the Matlab program to magnify the spectrum. Thus, in the spectrum is not seen the whole frequency range which is 2-7 Hz ,but only see the range 2-5 Hz due to the magnification.

As it was indicated before in **session 2** there are 3 data sets (**run1**, **run2**, **run3**) and the task is to identify where the sham stimulation was delivered (before recording **run2** or before recording **run3**). Based on the frequency differences across all channel groups it was possible to make a prediction of the likely stage (**run2** sham or **run3** sham) of pT-TMS in each of the 10 MS patients

In order to blindly identify real from sham stimulation it was necessary to predict the frequency increase due to pT-TMS from all recorded MEG channels. For this purpose it was needed to calculate the increase in primary dominant frequency from sham to real stimulation under two conditions. Having this in mind, one may estimate either the average frequency difference for each brain channel by calculating the differences between each average frequency of **(run1**+**run3**) / 2 from the **run2** if **run3** is the sham and **run2** is the real stimulation or the average frequency differences of (**run1**+**run2**) / 2 from the **run3** if the **run2** is the sham and **run3** is the real stimulation for the same patient in each brain channel as it is seen in the following equations:

$$\Delta f\,(2) = \text{run } 2 - (\text{run1}+\text{run3})/2 \qquad (1)$$

$$\Delta f\,(3) = \text{run3} - (\text{run1}+\text{run2})/2 \qquad (2)$$

In these equations **run1** is considered as the baseline MEG recordings, being the same for both calculations. In order to obtain all the above differences from all brain channels a software program was developed also in our laboratory (using eqs 1 and 2) to estimate the

mean peak frequency difference (MPFD) of ($\Delta f(2)$ or $\Delta f(3)$) for both calculations. If after all these calculations we have a MPFD from $\Delta(f(2)$ in (eq.1) to be greater for a particular patient then **run2** is the real stimulation and **run3** the sham stimulation or if the MPFD is greater from $\Delta f(3)$ in (eq.2) then **run3** is the real stimulation and the **run2** will be the sham stimulation.

Results

In this paper it was attempted to determine the order of stimulation (run2 sham or run3 sham)based on the MPFD as shown in **Table 1.** On each of the 10 MS patients our predictions were based (run2 sham or run3 sham) on whichever order gave rise to the largest change in the MPFD from all recorded channels.

In **Table 1** based on the knowledge of the true stimulation sequence, it is seen the true effect of pT stimulation. The largest Mean values indicate that our prediction for these MS patients was correct (in 9/10 cases). Based on the binomial test, the probability for correctly selecting 9 or more events, each with a probability of 0.5, from 10 by chance is $p \leq 0.01$ or chance level (90%).

The application of pT-TMS literature suggests that the real stimulus runs should have a higher frequency than the sham runs. This was correct in our case after unblinding as it is shown in **Table 1**. **Table 2** shows the brain regions and the corresponding channels in each brain region. **Table 3** shows the symptoms in each of the 10 MS patients after the sham stimulation as were evaluated in interviews by clinicians the next day after the sham stimulation (2nd day in our lab) , whereas **Table 4** shows the symptoms in each of the 10 MS patients evaluated by clinicians at the end of one month of daily pT-TMS treatment at home.

In order to determine the maximum effect of stimulation for each of the seven brain regions we have based our results to the maximum on the MPFD for all the 10 MS patients. Thus, in **Table 5,** are shown the MPFD in real and sham stimulation in Hz for each of the seven brain regions as it is stated in **Table 2** for all 10 MS Patients.

In addition, we have included three more **Tables 6,7 and 8** in order to explain our calculations. In each of these tables we have four columns. The first column, we have the channels of the chosen Right Temporal brain region(**Table 2**). In the second column, we have shown the calculations for the MEG recordings for the MS patient 1, using a software program developed in our laboratory, for run1(theta) of the base line. In the third column, we have shown the calculations for the run2(theta) and the third column for the run3(theta) in the band 2-7Hz and using the equation 1, if run2 is the real stimulation and run3 the sham stimulation, after the prediction from the unblinding process. We have also calculated the average and the standard deviations for the run1,run2 and run3 for the band 2-7Hz.

Discussion

There are only few studies in the literature concerning MEG and MS. Tewarie et al (2015) investigated the thalamo-cortical system to explain the presence of physical and cognitive problems in MS by means of functional magnetic resonance imaging (fMRI) and MEG. They concluded that thalamic atrophy is associated with global disruption of cortical functional networks in MS that was related to worse cognitive and clinical function in MS. Tewarie et al (2014) in a study with MEG computed the minimum spanning tree, a sub-graph of the original network and detect network changes in MS patients. These changes, such as a loss of hierarchical structure, are related to cognitive performance in MS. Schoonheim et al (2013) investigated functional connectivity changes in MS using MEG. They found specific functional changes in MS. Hardmeier et al (2012) explored the relationship of cognitive

performance to patterns of nodal centrality derived from MEG. They found that partial functional disconnection of the temporal regions was associated with cognitive dysfunction in MS. Tecchio et al (2008) investigated by MEG the intra-cortical connectivity in MS. Kotini et al. (2007a) showed that chaotic activity of MS patients is lower than in the normal subjects. Nonlinear analysis may offer fertile perspectives for understanding the features of patients with MS. Kotini et al (2007b) showed that some of the MEG recorded points in MS patients exhibited abnormal rhythmic activity, characterized by lower amplitudes and frequencies compared with controls. Using the MEG brain activity they were able to obtain a mapping technique characterized by the iso-contour spectral amplitude of scalp distribution. Cover et al (2006) in a study with MEG showed a decrease of the inter-hemispheric coherence measure in the MS patients, particularly in the alpha band which is in agreement with a reduced long-range connectivity in the brains of MS patients. Kassubek et al (1999) investigated by MEG patients with MS in order to find if abnormal cortical activity is associated with subcortical MS lesions using simultaneous bilateral recording of MEG activity. They found that the standardized maximum concentrations of dipoles were significantly higher in the MS patients than in the healthy subjects both in the slow and beta wave analysis.

In this study we replicate the effects of the increased abnormal dominant frequencies of 2-7 Hz band due to the effect of the pT-TMS (Anninos et al., 2015, 2008, 2007a, b, 2006, 2003, 2000, 1999, 1991, 1989; Kotini and Anninos, 2016) in a group of 10 MS patients.

The time frame of our clinical investigations was as follows:

1st day: MEG measurements in our lab (baseline run1). Application of sham stimulation and MEG recordings afterwards (run3). We found no significant differences in the patients' MEG spectrum.

2nd day: Interview by clinicians after the sham stimulation (Table 3). Application of real pT-TMS and MEG recordings afterwards (run2). The patients' MEG spectrum was almost like normal in the majority of them with absence most of the abnormal frequencies.

3rd day: Interview by clinicians after real stimulation. They confirmed our findings of our MEG recordings.

10th day: MEG recordings and evaluation by clinicians. Most of the patients reported a progressive deterioration of their pretreatment status.

To confirm that the responses to pT-TMS were reproducible we have advised the relatives of all MS patients to apply the pT-TMS treatment with the electronic device, mentioned before in the methods, with the same characteristics for each patient with those used in our laboratory, at home(23:00pm) every night. The instructions given to their relatives were as follows:

1. Place the helmet of the device on the patient head.

2. Turn the power switch on of the electronic device which is calibrated to produce pT-TMS with the characteristics of each MS patient for two minutes. This is indicated by a green light.

3. When the green light of the electronic device is turned off , turn the power switch off.

4. Remove the helmet from the head of the patient.

5. Each MS patient should turn off all the lights in the room and should go to bed immediately after treatment.

6. Store the electronic device in safe dry area.

Note that all electronic devices are operated with 4X1.5V batteries and all were new at the time that have given to MS patients for the above use.

After one month pT-TMS treatment at home all the MS patients were evaluated again and they all reported to have benefit from this treatment (Table 4). The mechanisms by which the application of the pT-TMS attenuated in the MS patient's syndrome are unknown. However one possible explanation is that these magnetic fields have been shown to influence the activity of the pineal gland (PG) which regulate the endogenous opioid functions (Lissoni et al 1986) and the dopaminergic modulation (Brandbury et al 1985), GABA (Nitsche et al 2006). Moreover on the cellular level, magnetic fields have shown to influence the properties and stability of biological membranes as well as their transport characteristics including the intra and extracellular distributions and flux of calcium ions (Ossenkopp and Cain 1988).

Conclusion

Therefore, it is possible to conclude that this method of the pT-TMS has some potential effect to be an important non invasive safe and efficacious modality in the management of MS patients. However, further investigations with more patients are necessary in order to evaluate its possible beneficial contribution for managing the symptoms of MS patients.

Conflicts of interest

None

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Table 1.This Table is shown the prediction to determine the order of stimulation (run2 sham or run3 sham) based on the mean peak frequency difference(MPFD). On each of the 10 multiple sclerosis patients the prediction was based (run2 sham or run3 sham) on whichever order gave rise to the largest change in the mean peak frequency difference(MPFD) from al MEG recorded channels. In patient 5 the MPFD was not clear and after unbliding the prediction was correct in 9/10(90%)

Patients Code	Run2	Run3	MPFD
1	Real stimulation	Sham stimulation	0.241>-0.248
2	Real stimulation	Sham stimulation	0.260>-1.175
3	Sham stimulation	Real stimulation	-1.567<0.884
4	Sham stimulation	Real stimulation	-0.384<0.021
5	Sham stimulation	Real stimulation	No clear
6	Real stimulation	Sham stimulation	0.981>-1.314
7	Sham stimulation	Real stimulation	-0.628<1.868
8	Sham stimulation	Real stimulation	-0.808<0.171
9	Real stimulation	Sham stimulation	0.313>-0.895
10	Sham stimulation	Real stimulation	-0.733<1.101
Prediction 90%			

Table 2. This table shows the brain regions and the corresponding channels in each brain region.

Brain Regions	Channels
Right Temporal	1-14 and 111-120
Right Parietal	5-6,11-16,97-100, 109, 110 and 115-122
Frontal	17-42
Left Temporal	43-50 and 55-62,67-74
Occipital	75-86,91-96 and 101-110
Vertex	13-16,49-54,61-66,73,74,89,90,99,100 and 117-122
Left Parietal	47-52,59-64,71-74,79,80,87-90

Table 3. This Table shows the symptoms of 10 MS patients before pT-TMS and after sham stimulation as were evaluated by interview by clinicians the next day after sham stimulation (2nd day in our lab)

Patients	Symptoms before pT-TMS	Symptoms after Sham stimulation
1	**Pyramidal Functions:** 1.Abnormal signs without disability. **Cerebellar Functions:** 3.Limb ataxia **Brain Stem Functions:** 5.Inability to shallow or to speak **Sensory Functions:** 3.Moderate decrease in touch **Bowel and Bladder Functions:** Mild urinary	**Pyramidal Functions:** No effect **Cerebellar Functions:** No effect **Brain Stem Functions:** No effect **Sensory Functions:** No effect **Bowel and Bladder Functions:** No effect **Visual Functions:** No effect **Cerebral Functions:** No effect

	hesitancy **Visual Functions:** 3.moderate decrease in fields **Cerebral Functions:** Mild decrease in mentation	
2	**Pyramidal Functions:** 1.Abnormal signs without disability. **Cerebellar Functions:** 1.Abnormal signs without disability **Brain Stem Functions:** 5.Inability to speak **Sensory Functions:** 3.Moderate decrease in pain **Bowel and Bladder Functions:** Mild urinary hesitancy **Visual Functions:** 0.Normal **Cerebral Functions:** 1.Mood alteration only	**Pyramidal Functions:** No effect **Cerebellar Functions:** No effect **Brain Stem Functions:** No effect **Sensory Functions:** No effect **Bowel and Bladder Functions:** No effect **Visual Functions:** Normal **Cerebral Functions:**No effect
3	**Pyramidal Functions:** 1.Abnormal signs without disability. **Cerebellar Functions:** 1.Abnormal signs without disability **Brain Stem Functions:** 5.Inability to speak **Sensory Functions:** 3.Moderate decrease in pain **Bowel and Bladder Functions:** Mild urinary hesitancy **Visual Functions:** 0.Normal **Cerebral Functions:** 1.Mood alteration only	**Pyramidal Functions: No effect** **Cerebellar Functions:** No effect **Brain Stem Functions:** No effect **Sensory Functions:** No effect **Bowel and Bladder Functions:** No effect **Visual Functions:** Normal **Cerebral Functions:** No effect
4	**Pyramidal Functions:** 2.Minimal disability **Cerebellar Functions:** 2.Mild ataxia **Brain Stem Functions:** 5.Inability to speak **Sensory Functions:** 3.Moderate decrease in position **Bowel and Bladder Functions:** Mild urinary hesitancy	**Pyramidal Functions:** No effect **Cerebellar Functions:** No effect **Brain Stem Functions:** No effect **Sensory Functions:** No effect **Bowel and Bladder Functions:** No effect **Visual Functions:** Normal **Cerebral Functions:** No effect

	Visual Functions: 0.Normal **Cerebral Functions:** 2.Mild decrease in mentation	
5	**Pyramidal Functions:** 2. Minimal disability. **Cerebellar Functions:** 2.Mild ataxia **Brain Stem Functions:** 5.Inability to swallow **Sensory Functions:** 3.Moderate decrease in touch **Bowel and Bladder Functions:** Mild urinary hesitancy **Visual Functions:** 0.Normal **Cerebral Functions:** 1.Mood alteration only	**Pyramidal Functions:** No effect **Cerebellar Functions:** No effect **Brain Stem Functions:**No effect **Sensory Functions:** No effect **Bowel and Bladder Functions:** No effect **Visual Functions:** Normal **Cerebral Functions:** No effect
6	**Pyramidal Functions:** 1.Abnormal signs without disability. **Cerebellar Functions:** 1.Abnormal signs without disability **Brain Stem Functions:** 5.Inability to swallow **Sensory Functions:** 3.Moderate decrease in position **Bowel and Bladder Functions:** Mild urinary retention **Visual Functions:** 0.Normal **Cerebral Functions:** 2.Mild decrease in mentation	**Pyramidal Functions:** No effect **Cerebellar Functions:** No effect **Brain Stem Functions:** No effect **Sensory Functions:** No effect **Bowel and Bladder Functions:** No effect **Visual Functions:** Normal **Cerebral Functions:** No effect
7	**Pyramidal Functions:** 2.Minimal disability. **Cerebellar Functions:** 2.Mild ataxia **Brain Stem Functions:** 5.Inability to speak **Sensory Functions:** 3.Moderate decrease in position **Bowel and Bladder Functions:** Mild urinary retention **Visual Functions:** 0.Normal **Cerebral Functions:** 2.Mild decrease in mentation	**Pyramidal Functions:** No effect **Cerebellar Functions:** No effect **Brain Stem Functions:** No effect **Sensory Functions:** No effect **Bowel and Bladder Functions:** No effect **Visual Functions:** Normal **Cerebral Functions:**No effect

8	**Pyramidal Functions:** 3.Mild paraparesis. **Cerebellar Functions:** 1.Abnormal signs without disability **Brain Stem Functions:** 5.Inability to speak **Sensory Functions:** 3.Moderate decrease in position **Bowel and Bladder Functions:** Mild urinary hesitancy **Visual Functions:** 0.Normal **Cerebral Functions:** 1.Mood alteration only	**Pyramidal Functions:** No effect **Cerebellar Functions:** No effect **Brain Stem Functions:** No effect **Sensory Functions:** No effect **Bowel and Bladder Functions:** No effect **Visual Functions:** Normal **Cerebral Functions:** No effect
9	**Pyramidal Functions:** 1.Abnormal signs without disability. **Cerebellar Functions:** 1.Abnormal signs without disability **Brain Stem Functions:** 5.Inability to speak **Sensory Functions:** 3.Moderate decrease in touch **Bowel and Bladder Functions:** Mild urinary urgency **Visual Functions:** 0.Normal **Cerebral Functions:** 1.Mood alteration only	**Pyramidal Functions:** No effect **Cerebellar Functions:** No effect **Brain Stem Functions:** No effect **Sensory Functions:** No effect **Bowel and Bladder Functions:** No effect **Visual Functions:** Normal **Cerebral Functions:** No effect
10	**Pyramidal Functions:** 2.Minimal disability. **Cerebellar Functions:** 2.Mild ataxia **Brain Stem Functions:** 2.Moderate nystagmus **Sensory Functions:** 1.Figure writing decrease only **Bowel and Bladder Functions:** Mild urinary urgency **Visual Functions:** 0.Normal **Cerebral Functions:** 1.Mood alteration only	**Pyramidal Functions:** No effect **Cerebellar Functions:** No effect **Brain Stem Functions:** No effect **Sensory Functions:** No effect **Bowel and Bladder Functions:** No effect **Visual Functions:** Normal **Cerebral Functions:** No effect

Table 4. This Table shows the symptoms of 10 MS patients before and after pT-TMS as each was evaluated by interview by clinicians according to the expanded disability status scale (EDSS), at the end of one month of daily pT-TMS treatment at home (F:Female; M:Male)

Patients	Sex	Symptoms before pT-TMS	Symptoms after pT-TMS
1	**F**	**Pyramidal Functions:** 1.Abnormal signs without disability. **Cerebellar Functions:** 3.Limb ataxia **Brain Stem Functions:** 5.Inability to shallow or to speak **Sensory Functions:** 3.Moderate decrease in touch **Bowel and Bladder Functions:**1. Mild urinary hesitancy **Visual Functions:** 3.moderate decrease in fields **Cerebral Functions:** Mild decrease in mentation	**Pyramidal Functions:** 0.Normal **Cerebellar Functions:** 0.Normal **Brain Stem Functions:** 0.normal **Sensory Functions:** 0.normal **Bowel and Bladder Functions:** 0.Normal **Visual Functions:** 0.normal **Cerebral Functions:** 0.normal
2	**F**	**Pyramidal Functions:** 1.Abnormal signs without disability. **Cerebellar Functions:** 1.Abnormal signs without disability **Brain Stem Functions:** 5.Inability to speak **Sensory Functions:** 3.Moderate decrease in pain **Bowel and Bladder Functions:** 1. Mild urinary hesitancy **Visual Functions:** 0.Normal **Cerebral Functions:** 1.Mood alteration only	**Cerebral Functions:** 0.Normal **Cerebellar Functions:** 0.Normal **Brain Stem Functions:** 0.Normal **Sensory Functions:** 0.Normal **Bowel and Bladder Functions:** 0.Normal **Visual Functions:** 0.Normal **Cerebral Functions:**0.Normal

3	**M**	**Pyramidal Functions:** 2.Minimal disability **Cerebellar Functions:** 2.Mild ataxia **Brain Stem Functions:** 5.Inability to speak **Sensory Functions:** 3.Moderate decrease in position **Bowel and Bladder Functions: 1.**Mild urinary hesitancy **Visual Functions:** 0.Normal **Cerebral Functions:** 2.Mild decrease in mentation	**Pyramidal Functions:** 0.Normal **Cerebellar Functions:** 0.Normal **Brain Stem Functions:** 0.Normal **Sensory Functions:** 0.Normal **Bowel and Bladder Functions:** 0.Normal **Visual Functions:** 0.Normal **Cerebral Functions:** 0.Normal
4	**F**	**Pyramidal Functions:** 2.Minimal disability. **Cerebellar Functions:** 2.Mild ataxia **Brain Stem Functions:** 5.Inability to swallow **Sensory Functions:** 3.Moderate decrease in pain **Bowel and Bladder Functions: 1.**Mild urinary hesitancy **Visual Functions:** 0.Normal **Cerebral Functions:** 1.Mood alteration only	**Pyramidal Functions:** 0.Normal **Cerebellar Functions:** 0.Normal **Brain Stem Functions:** 0.Normal **Sensory Functions:** 0.Normal **Bowel and Bladder Functions:** 0.Normal **Visual Functions:** 0.Normal **Cerebral Functions:**0.Normal
5	**F**	**Pyramidal Functions:** 2. Minimal disability. **Cerebellar Functions:** 2.Mild ataxia **Brain Stem Functions:** 5.Inability to swallow **Sensory Functions:** 3.Moderate decrease in touch **Bowel and Bladder Functions:** Mild urinary hesitancy **Visual Functions:** 0.Normal **Cerebral Functions:** 1.Mood alteration only	**Pyramidal Functions:** 0.Normal **Cerebellar Functions:** 0.Normal **Brain Stem Functions:** 0.Normal **Sensory Functions:** 0.Normal **Bowel and Bladder Functions:** 0.Normal **Visual Functions:** 0.Normal **Cerebral Functions:** 0.Normal

6	**F**	**Pyramidal Functions:** 1.Abnormal signs without disability. **Cerebellar Functions:** 1.Abnormal signs without disability **Brain Stem Functions:** 5.Inability to swallow **Sensory Functions:** 3.Moderate decrease in position **Bowel and Bladder Functions:** Mild urinary retention **Visual Functions:** 0.Normal **Cerebral Functions:** 2.Mild decrease in mentation	**Pyramidal Functions:** 0.Normal **Cerebellar Functions:** 0.Normal **Brain Stem Functions:** 0.Normal **Sensory Functions:** 0.Normal **Bowel and Bladder Functions:** 0.Normal **Visual Functions:** 0.Normal **Cerebral Functions:** 0.Normal
7	**F**	**Pyramidal Functions:** 2.Minimal disability. **Cerebellar Functions** 2.Mild ataxia **Brain Stem Functions:** 5.Inability to speak **Sensory Functions:** 3.Moderate decrease in position **Bowel and Bladder Functions:** 2. Mild urinary retention **Visual Functions:** 0.Normal **Cerebral Functions:** 2.Mild decrease in mentation	**Pyramidal Functions:** 0.Normal **Cerebellar Functions:** 0.Normal **Brain Stem Functions:** 0.Normal **Sensory Functions:** 0.Normal **Bowel and Bladder Functions:** 0.Normal **Visual Functions:** 0.Normal **Cerebral Functions:** 0.Normal
8	**M**	**Pyramidal Functions:** 3.Mild paraparesis **Cerebellar Functions:** 1.Abnormal signs without disability **Brain Stem Functions:** 5.Inability to speak **Sensory Functions:** 3.Moderate decrease in position **Bowel and Bladder Functions:3.**Mild urinary hesitancy **Visual Functions:** 0.Normal **Cerebral Functions:** 1.Mood alteration	**Pyramidal Functions:** 0.Normal **Cerebellar Functions:** 0.Normal **Brain Stem Functions:** 0.Normal **Sensory Functions:** 0.Normal **Bowel and Bladder Functions:** 0.Normal **Visual Functions:** 0.Normal **Cerebral Functions:** 0.Normal

		only	
9	**F**	**Pyramidal Functions:** 1.Abnormal signs without disability. **Cerebellar Functions:** 1.Abnormal signs without disability **Brain Stem Functions:** 5.Inability to speak **Sensory Functions:** 3.Moderate decrease in touch **Bowel and Bladder Functions:** 8.Mild urinary urgency **Visual Functions:** 0.Normal **Cerebral Functions:** 1.Mood alteration only	**Pyramidal Functions:** 0.Normal **Cerebellar Functions:** 0.Normal **Brain Stem Functions:** 0.Normal **Sensory Functions:** 0.Normal **Bowel and Bladder Functions:** 0.Normal **Visual Functions:** 0.Normal **Cerebral Functions:** 0.Normal
10	**F**	**Pyramidal Functions:** 2.Minimal disability. **Cerebellar Functions:** 2.Mild ataxia **Brain Stem Functions:** 2.Moderate nystagmus **Sensory Functions:** 1.Figure writing decrease only **Bowel and Bladder Functions:**9. Mild urinary urgency **Visual Functions:** 0.Normal **Cerebral Functions:** 1.Mood alteration only	**Pyramidal Functions:** 0.Normal **Cerebellar Functions:** 0.Normal **Brain Stem Functions:** 0.Normal **Sensory Functions:** 0.Normal **Bowel and Bladder Functions:** 0.Normal **Visual Functions:** 0.Normal **Cerebral Functions:**0.Normal

Table 5. This Table is shown the maximum effect of the MPFD in real and sham stimulations for each of the 10 patients as is indicated in Table 1. The fist column P is for the patient number, in the other columns the RT is for the right temporal brain region, the LT is for the left temporal brain region, the RP is for the right parietal region, the LP is for the left parietal region, the F is for the frontal region the V is for the vertex region and the O is for the occipital brain region.

P	RT Run2	RT Run3	LT Run2	LT Run3	RP Run2	RP Run3	LP Run2	LP Run3	F Run2	F Run3	V Run2	V Run3	O Run2	O Run3
1	5.63	2.75	5.63	1.75	4.88	2.5	3.38	2.63	5.5	1.13	3.38	2.63	5.75	0.63
2	5.03	2.6	5.44	5.44	5.03	2.66	5.2	5.3	5.3	5.2	5.2	5.3	4.03	3.38
3	2.4	2.72	2.34	3.72	4.81	5.3	1.88	4.53	3.63	4.97	5.47	5.25	2.88	3.53
4	3.13	5.34	4.56	5.6	3.13	4.78	4.91	3.38	4.22	3.03	5.1	3.76	2.47	3.28
5	3.81	4.34	3.78	5.41	3.81	5.31	2.16	5.41	3.94	2.1	2.1	3.93	3.25	5.31
6	5.63	3.94	5.00	2.19	5.63	3.94	5.13	2.19	2.94	0.88	5.13	3.94	5.38	0.81
7	2.16	5.31	3.53	5.47	2.84	5.28	3.53	5.41	2.66	5.44	2.53	5.41	2.84	5.47
8	2.69	3.63	3.1	3.56	1.94	3.63	3.31	4.5	4.75	2.75	3.31	3.25	4.75	5.19
9	4.81	1.94	4.00	0.56	4.31	4.56	4.00	4.19	2.88	2.88	4.81	4.56	4.88	5.31
10	3.91	4.06	1.69	4.66	4.34	4.44	2.88	4.81	3.53	4.22	1.69	5.19	4.28	5.22

Table 6 for Patient 1. In this Table is shown the calculated numbers for the run1 of the baseline in the band 2-7Hz,the run2 and run3 for the same band 2-7Hz which we have calculated using the equation 1 if run2 is the real stimulation and run3 the sham stimulation after the prediction from the unbinding process, stated before in the Methods. In this Table 6 is shown also the cannels of the Right Temporal brain region which was chosen to explain our calculations. From these numbers we are shown also the statistics for the average of the baseline run1, the real(run2 for theta) and sham(run3 for theta) and the corresponding standard deviations(SD). In this Table also is shown the maximum values for the MPFD which are 5.63Hz if run2 is the real stimulation and 2.75 if run3 is the sham stimulation which were shown also for patient 1 in Table 5. Av(run1 base line theta)=(3.24±1.31)Hz; Av(run2 theta)=(-0.66±2.48)Hz; Av(run3 theta)=(-0.08±1.49)Hz

Channels	Run1(theta)Hz	Run2(theta)Hz	Run3(theta)Hz
1	2.19	1.00	-0.31
2	2.31	-0.13	0.063
3	2.13	5.63	-2.81
4	2.00	0.44	-0.50
5	6.31	0.88	-0.44
6	2.81	-2.75	1.38
7	2.25	1.00	-0.50
8	3.06	-0.50	0.25
9	2.69	-0.75	0.38
10	3.25	-1.13	0.56
11	3.44	-0.06	0.13
12	6.50	-2.50	1.25
13	2.94	-0.63	0.69
14	2.13	1.38	-0.69
111	4.31	-2.13	1.06
112	2.00	4.00	-2.00
113	4.69	-1.25	-1.25
114	2.69	-5.50	2.75
115	2.31	-2.81	-2.63
116	4.50	1.19	-1.00
117	2.44	-2.38	-2.75
118	4.38	-2.13	1.06
119	2.13	-5.00	2.50
120	4.44	-1.75	0.88

Table 7 for Patient 2. In this Table is shown the calculated numbers for the run1 of the baseline in the band 2-7Hz,the run2 and run3 for the same band 2-7Hz which we have calculated using the equation 1 if run2 is the real stimulation and run3 the sham stimulation after the prediction from the unbinding process, stated before in the Methods. In this Table 7 is shown also the cannels of the Right Temporal brain region which was chosen to explain our calculations. From these numbers we are shown also the statistics for the average of the baseline run1, the real(run2 for theta) and sham(run3 for theta) and the corresponding standard deviations(SD). In this Table also is shown the maximum values for the MPFD which are 5.03Hz if run2 is the real stimulation and 2.6 if run3 is the sham stimulation which were shown also for patient 2 in Table 5. Av(run1 baseline theta)=(4.73±2.14)Hz; Av(run2 theta)=(0.64±2.64)Hz; Av(run3 theta)=(-2.39±2.08)Hz

Channels	Run1(theta)	Run2(theta)	Run3(theta)
1	6.94	1.88	-4.31
2	2.06	-2.22	-2.31
3	7.00	2.38	-4.19
4	2.75	-3.47	-1.69
5	2.81	0.34	-1.44
6	6.13	0.31	-3.25
7	2.81	2.41	2.59
8	2.88	-1.94	-2.03
9	7.00	-2.78	-1.47
10	2.13	4.38	-1.34
11	6.31	0.88	-3.53
12	2.13	1.81	-4.00
13	6.75	-2.13	-2.50
14	2.38	5.03	-1.91
111	7.00	-1.97	1.69
112	2.81	4.25	-1.84
113	3.00	-3.63	1.81
114	6.94	2.63	-4.13
115	6.94	2.66	-5.03
116	6.94	1.66	-4.16
117	6.69	2.56	-3.91
118	2.44	-2.78	-1.94
119	6.88	2.81	-5.44
120	3.94	0.34	-3.22

Table 8 for Patient 6. In this Table is shown the calculated numbers for the run1 of the baseline in the band 2-7Hz,the run2 and run3 for the same band 2-7Hz which we have calculated using the equation 1 if run2 is the real stimulation and run3 the sham stimulation after the prediction from the unbinding process, stated before in the Methods. In this Table is shown also the cannels of the Right Temporal brain region which was chosen to explain our calculations. From these numbers we are shown also the statistics for the average of the baseline run1, the real(run2 for theta) and sham(run3 for theta) and the corresponding standard deviations(SD). In this Table also is shown the maximum values for the MPFD which are 5.63Hz if run2 is the real stimulation and 3.94 if run3 is the sham stimulation which were shown also for patient 6 in Table 5. Av(run1 baseline theta)=(4.48±1.38)Hz; Av(run2 theta)=(0.31±2.40) Hz; Av(run3 theta)=(-0,97±2.30)Hz

Channels	Run1(theta)Hz	Run2(theta)	Run3(theta)
1	5.94	2.69	-4.06
2	3.44	-1.50	-0.94
3	5.94	3.69	-4.75
4	3.44	0.19	-0.38
5	6.69	-3.88	2.13
6	4.75	1.81	0.31
7	5.00	3.25	-3.69
8	4.75	-1.00	-1.00
9	3.44	2.50	-1.25
10	2.88	-1.50	-0.38
11	4.75	0.88	-1.94
12	4.75	-2.75	2.69
13	4.75	2.56	-2.31
14	4.75	-1.25	-1.25
111	2.38	-0.63	1.81
112	5.94	0.88	-2.13
113	5.94	-1.44	-2.19
114	5.94	0	1.31
115	5.94	-2.06	-1.69
116	2.19	5.63	-2.63
117	2.25	1.63	-4.94
118	2.19	2.44	-0.94
119	4.75	-2.25	3.94
120	4.75	-2.25	0.88

Figure 1. A) The 122 channel biomagnetometer SQUID inside the shielding room and a patient during MEG recording (small photo). B) The configuration of the stimulation coils within the helmet of the electronic device. C) The frequency output from the electronic device which has calibrated to 9Hz.

Figure 2. A) An MEG record of 8 sec obtained from a patient from which in B) after FFT analysis the primary dominant frequency is 2.5 Hz.

Highlights

- Our experimental design to MS patients was double-blind
- We applied pico Tesla Transcranial Magnetic Stimulation (pT-TMS) to MS patients
- Our predictions were based on the true order of stimulation (Sham or Real)
- After pT-TMS it was found an increase of frequencies in the range of 2-7 Hz
- Most of the MS patients reported benefit from the pT-TMS treatment

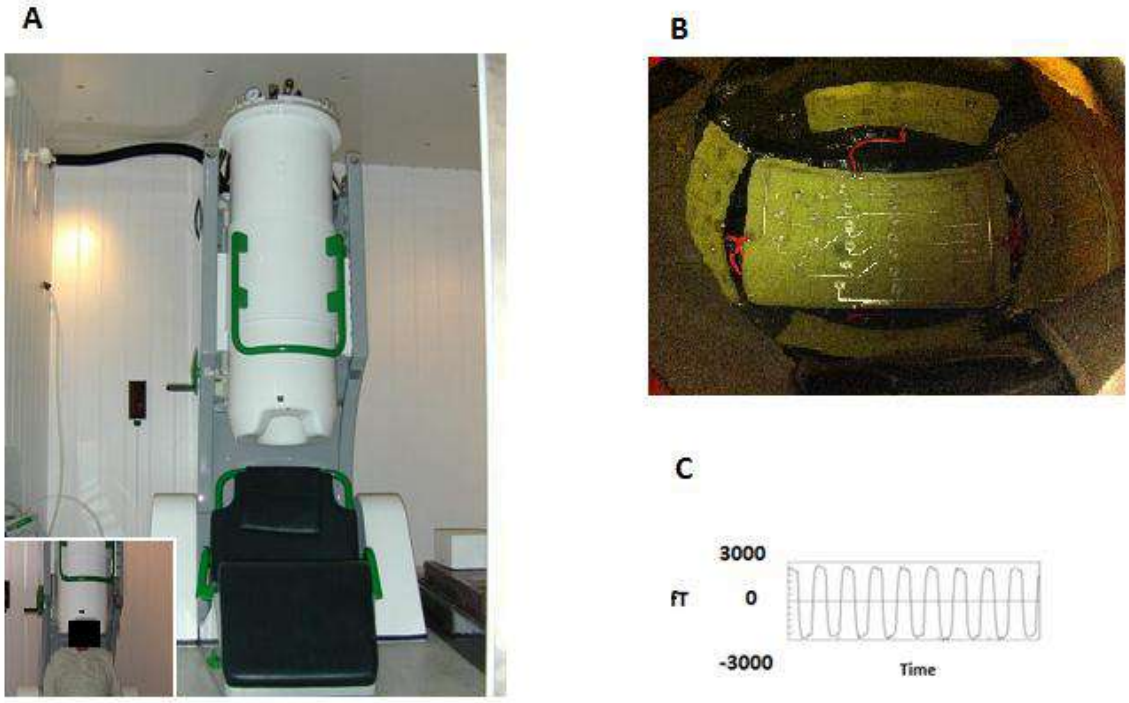

Fig.1

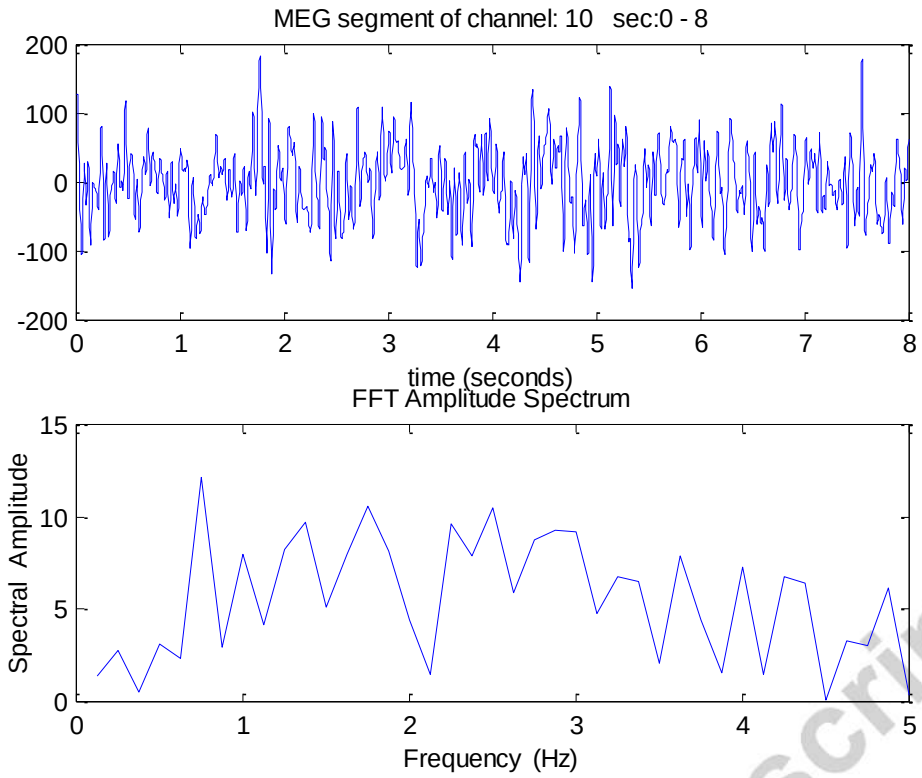

Fig. 2