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Research Article

Pico - Tesla External TMS on Dystonia Patients with a Double Blind Experimental Design. A MEG Study

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Abstract

Magnetoencephalographic (MEG) recordings of 10 dystonia patients (9 male,1 female) with ages ranging from 9 - 42 years were obtained using a whole - head 122 channel MEG system using a double - blind experimental design. External pico Tesla Transcranial Magnetic Stimulation (pT-TMS) was applied with magnetic field amplitude (1 - 7.5 pT) and frequency the alpha - rhythm of the patient (8 - 13Hz). We found a significant effect of an increase in the 2 - 7Hz frequencies range toward the patients' alpha rhythm. The results were statistically significant at 6 out of 10 patients (60%). The pT-TMS seems to be an important non-invasive safe and efficacious modality in the management of idiopathic migraine patients. Of course, more studies and additional investigations using controlled and larger samples should be performed before firm conclusions can be drawn.

Keywords: *MEG; Dystonia; pT-TMS; Brain Frequencies; Double Blind*

Introduction

Transcranial magnetic stimulation (TMS) is a non - invasive physiological tool of stimulating the cortical neurons and it is safe to the patient [1]. There are enough studies in the literature investigating the effects of TMS on dystonia patients. Wagle Shukla and Vaillancourt [2] in a review article summarized the data gained with TMS in Parkinson's disease and dystonia, and place of interest the present challenges in the use of TMS . They concluded that TMS is a noninvasive technique for investigation of excitatory and inhibitory changes relevant to Parkinson's disease and dystonia and can be used to discover a variety of underlying treatment mechanisms. Quartarone [3] in a review article summarized the effects of TMS in dystonia. He concluded that TMS can potentially be used as a therapeutic technique to treat some forms of dystonia, such as focal hand dystonia, where pharmacological options or injections of botulinum toxin are often ineffective.

Anninos., *et al*. suggested that pico - Tesla (pT) (1pT= 10-12 T) external transcranial magnetic stimulation (pT-TMS) to patients has some quantifiable benefits. Specifically, using an electronic device [4] they were able to increase the abnormal (2 - 7Hz) frequencies of the brain activity towards frequencies of less than or equal to those frequencies of the alpha frequency range (8 - 13Hz) of each patient [5-16]. One possible electrophysiological explanation for the efficacy of pT-TMS has been provided by the proposed "Neural Net Model" [13] that suggests that pT-TMS causes a temporally modulated neuronal inhibition in regions exhibiting abnormal activity in the frequency range of 2 - 7Hz.

There are no other studies in the literature investigated the effects of pT-TMS on dystonia patients by means of MEG. Thus, the aim of this research was to identify any change in the state between brain and motor behavior in these patients.

Materials and Methods

Biomagnetic measurements were performed using a whole-head 122-channel MEG device (Neuromag-122, Neuromag Ltd. Helsinki, Finland) (Figure 1A). Recordings were taken in an electromagnetically shielded room. The spontaneous MEG recordings were taken with sampling frequency rate of 256Hz and associated Nyquist frequency of 128Hz, that 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.

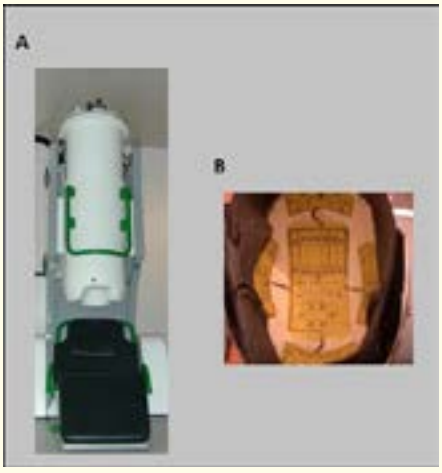

Figure 1: *A) The 122-channel MEG system. B) The pT-TMS electronic device.*

The dystonia patients were 10 (9 male, 1 female) with ages ranging from 9 - 42 years of age (mean: 14.7 ± 10.7). All had isolated dystonia at infancy (birth to 2 years). The patients were classified according to the American Classification of the Unified Dystonia Rating Scale, (UDRS) [17]. This research was approved by the Research Committee of the Democritus University of Thrace by a decision with a project number 80347. All patients were referred to our Laboratory of Medical Physics in Alexandroupoli, Greece, by practicing neurologists. In all patients informed consent for the methodology and the aim of the study was obtained prior to the procedure. In this study, we set out to show the effect of pT-TMS in dystonia patients using MEG recording protocols and a double - blind experimental design. In our study, we haven't included healthy subjects as controls because this research was already published by Troebinger., *et al*. [18] in which we have used also double - blind experimental design with our pico - Tesla electronic device [4] in order to look for an effect of pT-TMS on healthy subjects. All patients were at rest with their eyes closed in order to avoid artifacts and to enhance alpha rhythm during the MEG measurements. The head was stabilized within the MEG helmet with plastic patches. Four indicator coils were attached to the head of each 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. All MEG data tracings were visually inspected carefully off - line for movement artifacts and periods contaminated with movement artifacts were cut off.

The experimental protocol

The time taken for each MEG recording was 2 min 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 min MEG resting state scan. These data were consequently used to establish the subject's alpha frequency in the range of (8 - 13 Hz), for calibration of the pT-TMS electronic device.

In the second session (session 2), 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.

Initially, 2 min of pre-stimulus baseline MEG data were recorded (run1). After that 2 min of real or sham pT-TMS stimulation was administered with the subject sitting comfortably just outside the scanner room. Following the 2 min of stimulation, an extra of 2 min MEG of resting state data were acquired (run2). This was followed by a further of 2 min of stimulation-in this case the device was switched from sham to real or vice versa (by the third party)- and 2 additional min of MEG scanning data were carried out (run3).

The pT-TMS electronic device

The pT-TMS electronic device (Figure 1B) is a modified helmet containing up to 122 coils which are arranged in 5 array groups, so as to cover the 7 brain regions (frontal, vertex, right and left temporal, right and left parietal and occipital) for each subject. It is designed to create pT-TMS range modulations of magnetic flux (intensity: 1 - 7.5 pT), in the alpha frequency range (8 - 13Hz) of every dystonia patient.

We used the alpha rhythm for the stimulation because it is the only physiological rhythm of the patient generated at the occipital lobe and because we have found that it is the only way to improve the abnormal activity of their brain.

The pT-TMS device was configured for each patient to generate a square wave activity (so as to resemble the firing activity of neurons in the brain). Besides, the electronic device has an additional 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.

Spectral estimates

A software program was developed in our lab in order to detect the amplitude of the primary dominant frequency of the power spectra of the MEG recordings obtained from each dystonia patient and channel after the application of Fast Fourier Transform (FFT). Then we looked for interest at (alpha: 8 - 13Hz) for calibration of the electronic device and (2 - 7Hz) for the analysis at the primary dominant frequency of the power spectra of the MEG recordings obtained from each patient and channel after the application of FFT. Afterwards we can see using FFT that the primary dominant frequency is 2.9Hz (Figure 2).

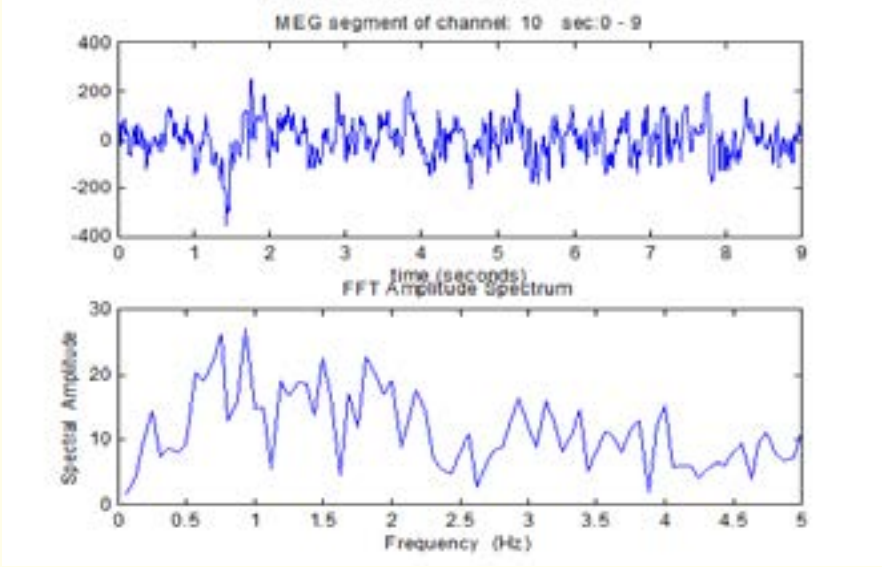

Figure 2: *This figure is shown the MEG record of 9 sec obtained from a dystonia patient with reference number 2. Application of FFT on the above MEG record we get the primary dominant frequency in the 2 - 7Hz which is in the range of 2.9 Hz.*

Analysis and Prediction of sham and stimulus runs

We performed our analysis as follows: first of all we have tried to blindly identify real from sham runs based on the predicted frequency increase due to pT-TMS.

As we have indicated, previously 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 we were able to make a prediction of the likely stage (run2 sham or run3 sham) of pT stimulation in each of the 10 recording MEG dystonia patients.

In order to blindly recognize real from sham stimulation, we have to predict the frequency, increase due to pT-TMS from all recorded MEG channels under two conditions. Thus, we can 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 dystonia patient in each brain channel as it is seen in the following equations 1 and 2.

$\Delta f(2) = run2 - (run1+run3)/2$ (1)

$\Delta f(3) = run3 - (run1+run2)/2$ (2)

In the above equations run1 is considered as the baseline MEG recordings. Lastly, after calculating the above differences from all brain channels we can estimate the mean peak frequency difference (MPFD) from ($\Delta f(2)$ or $\Delta f(3)$) .If after all these calculations we have a MPFD to be greater for a particular dystonia patient then run3 will be the real stimulation and run2 the sham stimulation or the run3 will be the sham stimulation and the run2 will be the real stimulation.

We used t - test for the statistical analysis of our results. The statistical significance was evaluated at the level of 0.05.

Results

Table 1 shows the symptoms in each of the 10 dystonia patients after the sham stimulation as they were evaluated in interviews by clinicians the next day after the sham stimulation (2nd day in our lab), and the symptoms in each of the patients evaluated by clinicians one month after daily pT-TMS treatment at home, following the instructions given to the relatives of all patients. We observed that 3 patients had no effect to pT-TMS and one patient had partially effected.

Patient code	Age	Sex	Symptoms before pT-TMS	Symptoms after Sham pT-TMS	Symptoms after pT-TMS
1	42	M	Segmental dystonia where two or more regions of the body are effected and the regions somehow are connected to each other as for example neck and shoulder. (UDRS:3)	No effect(UDRS:3)	He was partially effected in the neck and shoulder (UDRS:2)
2	9	M	Generalised dystonia where both legs and other regions of the body are effected. (UDRS:3)	No effect(UDRS:3)	He was feeling better(UDRS:0)
3	9	M	Focal dystonia where one part of the body is effected. (UDRS:3)	No effect(UDRS:3)	He was feeling better(UDRS:0)
4	12	M	Segmental dystonia where two or more regions of the body are effected and the regions somehow are connected to each other as for example neck and shoulder. (UDRS:3)	No effect(UDRS:3)	No effect(UDRS:2)

5	25	M	Secondary dystonia where the dystonia signs and symptoms occur as a consequence of an underlying condition - usually genetic, neurological or an injury which affects the nervous system. (UDRS:3)	No effect(UDRS:3)	He has a substantial effect(UDRS:0)
6	10	M	Focal dystonia where one part of the body is effected(UDRS:3)	No effect(UDRS:3)	No effect(UDRS:3)
7	10	M	Multifocal dystonia where at least two regions of the body that are not connected to each other are affected as for example in this case one arm on one side as well as one leg of the other side of the body. (UDRS:3)	No effect(UDRS:3)	He has effect in both sides of his body(UDRS:3)
8	12	M	Focal dystonia where one part of the body is effected (UDRS:3)	No effect(UDRS:3)	Very good effect(UDRS:0)
9	9	M	Generalised dystonia where both legs and other regions of the body are effected. (UDRS:3)	No effect(UDRS:3)	No effect(UDRS:3)
10	9	F	Focal dystonia where one part of the body is effected. (UDRS:3)	No effect(UDRS:3)	She has a good effect (UDRS:0)

Table 1: *This Table shows the symptoms of 10 dystonia patients in A) Interview from clinicians the next day after sham stimulation (2nd day in our lab) (According to the American Classification of the Unified Dystonia Rating Scale, (UDRS)) and B) The effect of pT-TMS as were evaluated by interview by clinicians (According to the American Classification of the Unified Dystonia Rating Scale, (UDRS)) one month after daily pT-TMS treatment at home (F: Female; M: Male)*

We have attempted to determine the order of stimulation (run2 sham or run3 sham) based on the MPFD as shown in Table 2. On each of the 10 dystonia patients we have based our predictions (run2 sham or run3 sham) on whichever order gave rise to the largest change in the MPFD from all MEG recorded channels. In Table 2, based on the information of the true stimulation sequence, we can show the true effect of pT-TMS. Based on the binomial test, the possibility for correctly selecting 9 or more events, each with a probability of 0.5, from 10 patients is highly statistical significant or at chance level (90%).

Patients Code	Run2	Run3	Average MPFD Hz
1	Sham stimulation	Real stimulation	- 0.073 < 0.718
2	Real stimulation	Sham stimulation	1.020 > - 0.575
3	Sham stimulation	Real stimulation	0.180 < 0.626
4	Real stimulation	Sham stimulation	0.693 > 0.070
5	Real stimulation	Sham stimulation	0.784 > - 0.370
6	Real stimulation	Sham stimulation	No clear
7	Real stimulation	Sham stimulation	2.049 > - 1.038
8	Real stimulation	Sham stimulation	1.388 > - 0.071
9	Real stimulation	Sham stimulation	1.344 > 0.328
10	Real stimulation	Sham stimulation	1.405 > - 0.497
Prediction 90%			

Table 2: *We show the prediction to determine the order of stimulation (run2 sham or run3 sham) based on the average MPFD in (2 - 7 Hz) from all channels as is described by eqs. 2, 3. On each patient the prediction was based (run2 sham or run3 sham) on any order gave rise to the largest change in average MPFD from all MEG recorded channels.*

The application of pT-TMS literature suggests that the real stimulus runs should have a higher frequency than the sham runs [4-16]. This was correct in our case after unblinding as we can see in Table 2. Table 3 shows the brain regions and the corresponding channels in each brain region. In order to determine the maximum effect of stimulation for each of the 7 brain regions we based our results to the maximum on the MPFD for all patients. Therefore, in Table 4 is shown the maximum MPFD in Hz from Real (run2 in Δf(2)) to Sham (run3 in Δf(3)) and Real (run3 in Δf(3)) to Sham (run2 in Δf(2)) stimulation correspondingly, for each of the 7 brain regions as it is stated in Table 3 for all patients. Table 5 shows the statistical analysis for the 10 dystonia patients. We observed a statistical significance difference at 6 out of 10 patients (60%).

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

Table 3: *It is shown the brain regions and the corresponding channels in each brain region.*

P	RT	RT	LT	LT	RP	RP	LP	LP	F	F	V	V	O	O
	Run2 Real MPFD	Run3 Sham MPFD	Run2 Real MPFD	Run3 Sham MPFD	Run2 Real MPFD	Run3 Sham MPFD	Run2 Real MPFD	Run3 Sham MPFD	Run2 Real MPFD	Run3 Sham MPFD	Run2 Real MPFD	Run3 Sham MPFD	Run2 Real MPFD	Run3 Sham MPFD
2	5.25	1.16	5.44	1.66	5.25	2.31	5.44	1.06	5.28	4.56	5.44	2.31	5.16	0.94
4	3.94	3.19	3.94	3.44	3.88	3.19	4.94	3.06	2.69	4.44	4.94	3.44	3.94	4.13
5	5.34	0.31	5.31	4	4.5	2.81	3.22	3.72	3.88	1	4.5	3.7	5.34	1.84
6	1.84	2.5	2.03	4	2	2.75	2.72	4	4	2	2.88	2.75	1.88	1.75
7	5.13	1.09	5.88	1.94	5.13	2.13	5.34	1.031	5.25	0.25	5.34	0.72	5.78	2.09
8	5.5	4.66	4.88	3.47	5.31	4.66	4.88	3.88	5.16	5.16	4.84	4.66	5.88	4.25
9	4.41	3.06	5.44	4.41	4.41	3.31	4	4.41	3.44	4.56	4.41	4	3.91	3.31
10	5.06	5.06	5.13	3.25	4.75	5	5.13	3.25	3.56	3.44	5.13	2.81	5.56	3
P	**RT**	**RT**	**LT**	**LT**	**RP**	**RP**	**LP**	**LP**	**F**	**F**	**V**	**V**	**O**	**O**
	Run3 Real MPFD	**Run2 Sham MPFD**	**Run3 Real MPFD**	**Run2 Sham MPFD**	**Run3 Real MPFD**	**Run2 Sham MPFD**	**Run3 Real MPFD**	**Run2 Sham MPFD**	**Run3 Real MPFD**	**Run2 Sham MPFD**	**Run3 Real MPFD**	**Run2 Sham MPFD**	**Run3 Real MPFD**	**Run2 Sham MPFD**
1	5.31	4.25	3.47	4.19	5.31	4.25	2.63	4.19	4.53	3.25	5.31	2.13	4.84	5.09
3	5.06	2.38	4.69	2.31	5.06	4.44	4.69	2	1.81	1.94	4.69	2.38	3.5	4.44

Table 4: *It is shown the effect of the maximum MPFD value in real and sham stimulations for each patient, according to the order of stimulation (run2 sham or run3 sham) in Table 2. (P is the patient number, RT is the right temporal brain region, LT the left temporal region, RP is the right parietal region, LP is the left parietal region, F is the frontal region, V is the vertex region and O is the occipital brain region).*

Patients	Run2 (REAL) Mean ± SD	Run3 (SHAM) Mean ± SD	t - test P values
2	5.34 ± 0.11	1.99 ± 1.27	0.0001
4	4.04 ± 0.76	3.56 ± 0.52	0.1611
5	4.59 ± 0.78	2.47 ± 1.46	0.0054
6	2.48 ± 0.78	2.82 ± 0.88	0.4453
7	5.41 ± 0.31	1.32 ± 0.74	0.0001
8	5.21 ± 0.39	4.39 ± 0.57	0.0086
9	4.29 ± 0.62	3.87 ± 0.62	0.1622
10	4.93 ± 0.63	3.69 ± 0.95	0.0139
Patients	**RUN3 (REAL) MEAN ± SD**	**RUN2 (SHAM) MEAN ± SD**	**t - test P values**
1	4.49 ± 1.05	3.91 ± 0.94	0.3166
3	4.23 ± 1.19	2.84 ± 1.11	0.0436

Table 5: *Statistical analysis for the dystonia patients of Table 4. The results are statistical significant at the level of 0.05 (marked bold).*

Discussion

Lozeron., *et al.* [19] in a review paper discussed the contribution of TMS and repetitive TMS in the pathophysiology and treatment of dystonia. They concluded that TMS is an important tool in understanding the pathophysiology of dystonia but large controlled studies using sham stimulation are still required to define the role of rTMS in its treatment.

In our research, we have replicated the effects of the increased abnormal dominant frequencies of 2 - 7 Hz by the application of pT-TMS. The time frame of our clinical investigations was in the following way:

- **1st day:** MEG measurements in our lab (baseline run1). Application of sham stimulation and MEG recordings afterwards (run3). We have found no significant differences in patients' MEG spectrum.
- **2nd day:** Interview by clinicians after the sham stimulation. Application of real pT-TMS and MEG recordings afterwards (run2). The patients' MEG spectrum was almost normal in the majority of the patients with absence most of the abnormal frequencies.
- **3rd day:** Interview by the same clinicians after real stimulation. They confirmed our findings of our MEG recordings.
- **10th day:** MEG recordings and evaluation by the same clinicians. Most of the patients reported a progressive deterioration of their pretreatment status.

As a final point to confirm that the responses to pT-TMS were reproducible we have advised the relatives of all dystonia patients to apply the pT-TMS treatment nightly at home (23:00 pm). After one month of pT-TMS treatment at home all the dystonia patients were evaluated again with MEG recordings and interview by the same clinicians and their benefit from this treatment as it is shown in Table 4.

The mechanism by which the application of the pT-TMS has some beneficial effects in the dystonia patients are unidentified. Although, one possible explanation is that these magnetic fields (pT-TMS) have been shown to influence the activity of the pineal gland (PG) which regulates the endogenous opioid functions [20] and the dopaminergic modulation [21], GABA [22]. Moreover, the PG is a regulator of our immune system through the action on the thymus gland generating the infection fighting T-cells which are needed to neutralize foreign invaders such as viruses and bacteria. If the thymus gland shrinks with the age or due to other disorders its ability to generate T-cells is sapping.

Conclusion

This method of the pT-TMS may be considered as a non-invasive safe and efficacious modality in managing the symptoms of dystonia patients where we have 60% effect of using the pT-TMS. The limitations of our study is the small number of patients and the absence of a control group due to the previous publication by Troebinger., *et al* [18]. This study is preliminary and additional investigations using controlled and larger samples should be performed before this approach could be adopted.

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Conflicts of Interest

None.

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