Journal of Integrative Neuroscience, Vol. 15, No. 3 (2016) 1–17

DOI: 10.1142/S0219635216500278

A combined study of MEG and pico-Tesla TMS on children with autism disorder

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[Received 21 December 2015; Accepted 14 October 2016; Published 23 November 2016]

Magnetoencephalographic (MEG) recordings from the brain of 10 children with autism (6 boys and 4 girls, with ages range from 5–12 years, mean ± SD: 8.3 ± 2.1) were obtained using a whole-head 122-channel MEG system in a magnetically shielded room of low magnetic noise. A double-blind experimental design was used in order to look for possible effect of external pico-Tesla Transcranial Magnetic Stimulation (pT-TMS). The pT-TMS was applied on the brain of the autistic children with proper field characteristics (magnetic field amplitude: 1–7.5 pT, frequency: the alpha — rhythm of the patient 8–13 Hz). 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 their MEG recordings. The statistical analysis of our results shown a statistical significance at 6 out of 10 patients (60%). It is also observed an increase of alpha activity in autistic children at the end of one month after pT-TMS treatment at home. In conclusion, the application of pT-TMS has the prospective to be a noninvasive, safe and important modality in the management of autism children.

Keywords: MEG; pT-TMS; autism children; double blind.

1. Introduction

Transcranial Magnetic Stimulation (TMS) is a technique which has a variety of diagnostic and therapeutic uses in neurological conditions such as Multiple Sclerosis (MS), Parkinson Disease (PD), epilepsy and other patients of brain disorders (Anninos *et al.*, 1991, 2000, 2006, 2007, 2008, 2016a,b; Chen *et al.*, 2008). It is safe, non-invasive method and was developed as an alternative to transcranial electrical simulation (Barker *et al.*, 1985). Frye *et al.* (2008) in a review article concluded that the potential for applications of TMS in child neurology and psychiatry is important.

Given its excellent safety profile and possible therapeutic effect, this technique should develop as a significant tool in pediatric neurology. Narayana *et al.* (2015) suggested that TMS is a noninvasive tool that is safe for use in children and adolescents for functional mapping and treatment, and for many children it aids in the preoperative assessment and the risk-benefit decision making. Garvey & Mall (2008) reported that TMS can potentially provide insights into both typical neuromotor maturation and the mechanisms underlying the motor skill deficits in children with developmental disabilities. Rajapakse & Kirton (2013) suggested that TMS represents an exciting advancement to better understand and improve outcomes from disorders of the developing brain. Anninos *et al.* (1991) demonstrated that MEG recordings in patients with seizure disorders show significant MEG activity often in the absence of Encencephalogram (EEG) abnormalities. Using external pT-TMS, we were able to successfully attenuate seizure activity in over 100 patients suffered with various forms of epilepsy. Similar studies Anninos *et al.* (2000) using MEG recordings [Fig. 1(a)] in PD patients observed that these recordings exhibited abnormal activities at high amplitudes and low frequencies in the range of 2–7 Hz. The application of pT-TMS with intensity 1–7.5pT and frequency the a-rhythm of the patient (8–13 Hz) for 2 min, in the left-right temporal, frontal-occipital and vertex and recorded the MEG activity again, resulted in a rapid attenuation of the MEG activity. Other studies Anninos *et al.* (2016a) using MEG recordings in MS patients observed that

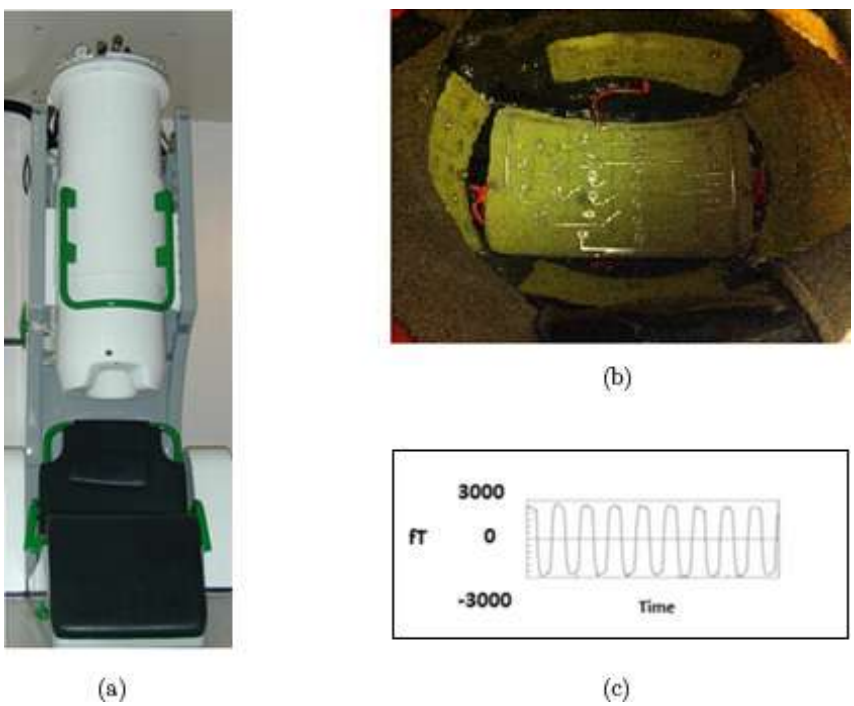


Fig. 1. (a) The configuration of the stimulation coils within the helmet of the electronic device. (b) The frequency output from the electronic device which has been calibrated to 9 Hz.

these recordings exhibited abnormal activities by high amplitudes and low frequencies in the range of 2–7 Hz. The application of pT-TMS with similar characteristics in the intensity and frequency which was used for the PD patients and recorded again the MEG activity, resulted in a rapid attenuation and normalization of the MEG activity. Other TMS studies in individuals with MS have shown variable sensitivities to clinical signs and symptoms (Curra *et al.*, 2002). Sandyk (1992b) referred to a patient with a history of chronic-progressive MS in whom extracranial application of pT-TMS produced a dramatic and sustained improvement in disability.

In our study we used our pico Tesla TMS (pT-TMS) electronic device (Anninos & Tsagas, 1995) for the therapeutic treatment of children suffering from Autism [Figs. 1(b) and 1(c)] because the higher level of TMS might have side effects to children. This device is a modified helmet containing up to 122 coils which have been 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 (8–13 Hz) of each patient (Anninos *et al.*, 2016a,b, 2015, 2008, 2007, 2006, 2003, 2000, 1999, 1991, 1989, 1986). We used only the a-rhythm because it is the physiological rhythm for each subject. The pT-TMS device is calibrated with the a-rhythm frequency of each patient and was configured for each individual patient to generate a square wave so as to resemble with the firing activity of neurons in the brain (Anninos *et al.*, 1970) [Figs. 1(b) and 1(c)]. We have tested also other frequencies as well as other forms of oscillations before but we didn't have any effects.

The electronic device has an extra hidden switch to disable current flow to the helmet coils. This switch, is for controlling real or sham stimulation 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).

2. Methods

Biomagnetic measurements were performed using a whole-head 122-channel MEG system (Neuromag-122, Neuromag Ltd. Helsinki, Finland) (Anninos *et al.*, 2016a,b, 2015, 2010, 2008, 2007; Kotini & Anninos, 2016; Kotini *et al.*, 2010, 2008, 2005) [Fig. 1(a)]. MEG recordings were taken in an electromagnetically shielded room in order to avoid extraneous electromagnetic noise. These recordings were taken with sampling frequency rate of 256 Hz and the associated Nyquist frequency of 128 Hz, was well above constituent frequency components of interest in our MEG recordings avoiding aliasing artifacts. The MEG signal was filtered with cut-off frequencies at 0.3 Hz and 40 Hz.

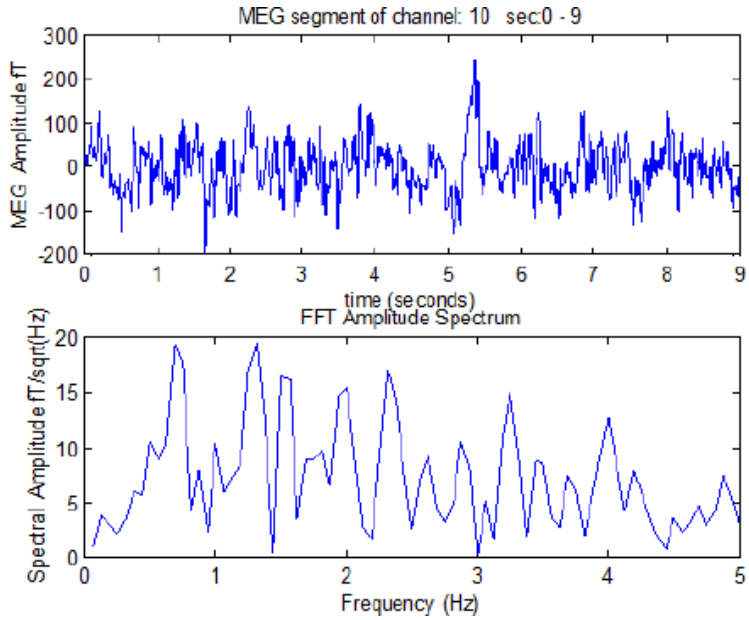
MEG recordings from the brain of 10 children with Autism (6 boys and 4 girls, with ages range from 5–12 years, mean $\pm$ SD: 8.3 ± 2.1) were obtained using the above-mentioned MEG.

This research was approved by the Research Committee of the Democritus University of Thrace by a decision with the project number 80347. All patients were

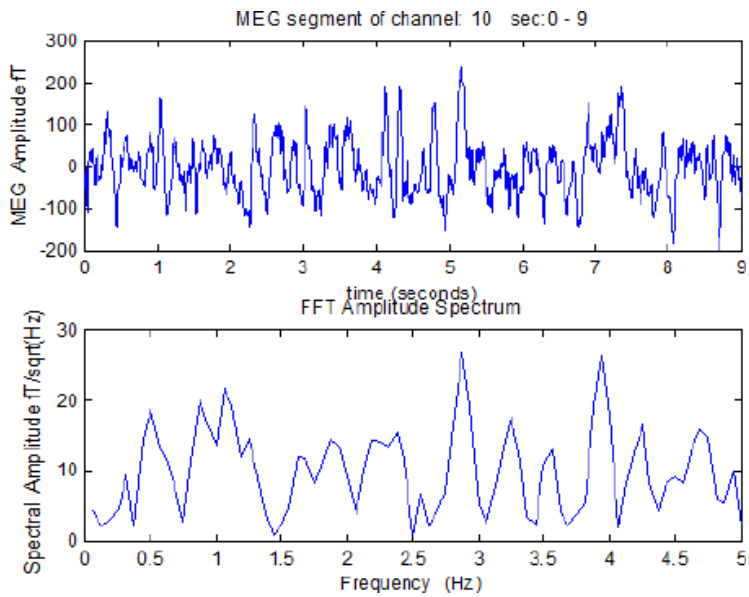
referred to our Laboratory of Medical Physics in Alexandroupoli, Greece, by practicing neurologists. For all patients, informed consent for the methodology and the aim of the study was obtained by their parents prior to the procedure. In this study, we set out to show the effect of pT-TMS on autistic disorder patients using MEG recordings protocol and a double-blind experimental design. In our study, we have not included healthy subjects as control because the research was already published by Troebinger *et al.* (2015) who have used a double-blind experimental design with our pT-TMS electronic device (Anninos & Tsagas, 1995) in order to look for an effect on healthy subjects. All the patients were in the same condition (at rest with eyes closed in order to avoid artifacts and to enhance alpha rhythm) during the MEG measurements. The patients head was stabilized within the MEG helmet by plastic patches, so as to reach maximal stabilization. In addition, 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 HPI digitizer. All MEG data tracings were visually inspected off-line for movement artifacts and periods contaminated with movement artifacts were cut off. The time taken for each 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 helped 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 [Figs. 1(b) and 1(c)]. 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 min of pre-stimulus baseline MEG data were recorded (run1). Next, 2 min of real or sham pT-TMS stimulation was administered with the subject sitting comfortably just outside the scanner room. Following these 2 min of (real or sham) stimulation, a further 2 min of resting state MEG data were acquired (run2). This was followed by another 2 min of stimulation but in this case the device was switched from sham to real or vice versa (by the third party)- and 2 more minute of MEG scanning data were carried out (run3).

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 autistic patient and channel after the application of Fast Fourier Transform (FFT) [Figs. 2(a) and 2(b)]. 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. Figures 2(a) and 2(b) are examples for the primary dominant frequency before and after the application of pT-TMS, where the actual signal length for analysis was 2 min and the FFT was applied only for 5 s, and in order to explain the primary



(a)



(b)

Fig. 2. The child with reference number 2. (a) The MEG record and the power spectra before pT-TMS. The primary dominant frequency in the 2–7 Hz band is 2.3 Hz. (b) The MEG record and the power spectra after pT-TMS. The primary dominant frequency in the 2–7 Hz band was moved to 2.9 Hz with another peak at 3.9 Hz.

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 but only the range 0–5 Hz due to magnification. As it was mentioned before in session 2 there are 3 data sets (run1, run2 and run3) and the task is to identify where the sham stimulation was delivered (before recording run2 or before recording run3). Thus, 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) or pT-TMS in each of the 10 autistic 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 from each autistic patient. 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 condition is to estimate 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 other condition is to estimate the average frequency differences of (run1+run2)/2 from the run3 if the run2 is the sham and the run3 is the real stimulation for the same patient in each brain channel as it is seen in the following equations:

$$\Delta f(2) = \text{run2} - (\text{run1} + \text{run3})/2, \tag{1}$$

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

In these equations run1 is considered as the baseline MEG recordings, being the same for both calculations. In order to obtain all of the above differences from all brain channels from each patient a software program was developed also in our laboratory using the above equations 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 the above equation to be greater for a particular patient then run2 is the real stimulation and the run3 is the sham stimulation or if the MPFD is greater from $\Delta(f(3))$ in the above equation then run3 is the real stimulation and the run2 will be the sham stimulation. The delivery of sham and real stimulation was not audible. After the application of pT-TMS there were also effects at the rest of the power spectrum but we have chosen the MPFD because the effect was more significant.

3. Results

As we have stated before in this paper it was attempted to determine the order of simulation (run2 sham or run3 sham) based on the MPFD as it is shown in Table 1.

On each of the 10 autistic patients our prediction 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 the pT stimulation. The largest Mean values

Table 1. This table shows the prediction to determine the order of stimulation (run2 sham or run3 sham) based on the average of all the MPFD in all brain channels in each patient in band 1 (2–7 Hz) as is described by Eqs. (2) and (3). On each of the 10 autistic children the prediction was based (run2 sham or run3 sham) on whichever order gave rise to the largest change in MPFD from all MEG recorded channels. In patient 10 the MPFD was not clear because the difference between the two average MPFD was too small to discriminate. After unblinding the prediction was correct in 9/10 (90%).

Patients Code	Run2	Run3	Average MPFD (Hz)
1	Sham stimulation	Real stimulation	−1.013<1.255
2	Real stimulation	Sham stimulation	1.173>0.448
3	Sham stimulation	Real stimulation	−0.160<0.473
4	Real stimulation	Sham stimulation	−0.497> −0.615
5	Real stimulation	Sham stimulation	0.496> −0.383
6	Sham stimulation	Real stimulation	−1.099<1.495
7	Sham stimulation	Real stimulation	−0.321<0.472
8	Real stimulation	Sham stimulation	1.167>0.459
9	Real stimulation	Sham stimulation	0.082> −1.286
10	Real stimulation	Sham stimulation	No clear

indicate that our prediction for these 10 autistic patients was correct in 9/10 cases 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.

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 autistic patients. Thus, in Tables 3 and 4 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 autistic patients.

Table 5 shows the symptoms in each of the 10 autistic children after the sham stimulation as were evaluated in interviews by clinicians the next day after the sham

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

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. This table is shows the maximum MPFD value from real and sham stimulations for each of the 2,4,5,8,9 and 10 of the 10 autistic patients, according to the order of stimulation (run2 sham or run3 sham) in Table 1. In this table in the first column P is for the patient number, in the other columns the RT is for the right temporal brain region, the LT for the Left temporal 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 for the occipital brain region.

P	RT Run2 Real MPFD	RT Run3 Sham MPFD	LT Run2 Real MPFD	LT Run3 Sham MPFD	RP Run2 Real MPFD	RP Run3 Sham MPFD	LP Run2 Real MPFD	LP Run3 Sham MPFD	F Run2 Real MPFD	F Run3 Sham MPFD	V Run2 Real MPFD	V Run3 Sham MPFD	O Run2 Real MPFD	O Run3 Sham MPFD
2	4.81	4.09	3.44	3.78	4.81	4.09	4.5	3.78	4.84	4.09	5.2	4.09	4.3	3.7
4	1.84	2.5	2.03	4.00	2.00	2.75	2.72	4.00	4.00	2.00	2.88	2.75	1.88	1.75
5	3.75	0.81	3.19	0.56	3.75	0.81	4.69	0.69	2.94	0.44	4.69	0.94	3.06	0.81
8	3.84	4.44	4.78	4.41	4.59	4.16	4.91	3.84	3.72	4.00	4.91	3.41	5.1	3.28
9	1.94	0.81	4	−0.31	1.94	0.81	4	0.56	3.63	0.63	4	0.81	3.75	0.56
10	0.59	0.94	0.97	2.00	0.88	2.10	1.50	2.00	3.75	4.03	3.00	1.63	0.47	−0.03

Table 4. This table shows the maximum MPFD value from Real (run3 in $\Delta(f(3))$ to Sham(run2 in $\Delta f(2)$) stimulations for each of the 1, 3, 6 and 7 from the 10 autistic patients, according to the order of stimulation (run2 sham or run3 sham) in Table 1. (P : patient number, RT: right temporal, LT : left temporal, RP : right parietal, LP : left parietal, F : frontal, V : vertex, O: occipital).

P	RT Run3 Real MPFD	RT Run2 Sham MPFD	LT Run3 Real MPFD	LT Run2 Sham MPFD	RP Run3 Real MPFD	RP Run2 Sham MPFD	LP Run3 Real MPFD	LP Run2 Sham MPFD	F Run3 Real MPFD	F Run2 Sham MPFD	V Run3 Real MPFD	V Run2 Sham MPFD	O Run3 Real MPFD	O Run2 Sham MPFD
1	−0.19	4.44	2.19	1.88	3.38	5.13	1.44	5.19	0.81	4.44	4.69	5.13	3.13	5.44
3	4.5	3.5	5.25	4.50	4.50	3.50	5.25	4.50	4.25	3.00	4.25	4.00	3.25	3.00
6	4.25	0.69	3.81	4.19	5.25	0.69	3.81	4.25	4.19	1.94	4.25	4.25	4.38	4.88
7	1.25	1.63	1.25	0.88	1.50	2.63	1.63	0.88	1.25	4.00	1.38	1.63	2.88	2.50

Table 5. This table shows the full-text of diagnostic criteria for Autism Spectrum Disorder (ASD) and the related diagnosis of Social Communication Disorder (SCD) as they appear in the 5th edition of Diagnostic and Manual of Mental Disorders (DSM-5) as of May 2013 and have evaluated by interview of the 10 autistics patients by neurology clinicians the next day in our Lab before and after sham pT-TMS.

Patient	Age	Sex	Symptoms Before pT-TMS	Symptoms After Sham pT-TMS
1	9	M	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	No effect
2	8	F	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	No effect
3	5	M	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	No effect
4	8	F	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	No effect
5	6	M	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	No effect

Table 5. (*Continued*)

Patient	Age	Sex	Symptoms Before pT-TMS	Symptoms After Sham pT-TMS
6	9	F	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	No effect
7	12	M	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	No effect
8	11	F	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	No effect
9	7	M	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	No effect
10	8	M	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	No effect

Table 6. This table shows the full-text of diagnostic criteria for ASD and the related diagnosis of SCD as they appear in the 5th edition of Diagnostic and Manual of Mental Disorders (DSM-5) as of May 2013 and have been evaluated by interview of the 10 autistics patients by neurology clinicians after one month treatment with pT-TMS at home.

Patient	Age	Sex	Symptoms Before pT-TMS	Symptoms After One Month with pT-TMS
1	9	M	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions, or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	Normal changes and behavior in the list of disorders
2	8	F	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions, or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	Normal changes and behavior in this list of disorders
3	5	M	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions, or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	Major changes in this list of disorders
4	8	F	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions, or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	Mixed changes in the list of disorders
5	6	M	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions, or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	Major changes in this list of disorders

Table 6. (*Continued*)

Patient	Age	Sex	Symptoms Before pT-TMS	Symptoms After One Month with pT-TMS
6	9	F	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions, or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	Minor Changes from the list of behaviors
7	12	M	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions, or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	Minor Changes from the list of behaviors
8	11	F	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions, or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	Major changes in this list of disorders
9	7	M	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions, or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	Major changes in this list of disorders
10	8	M	Persistent deficits in social communication and social interaction, as is manifested by the deficits in social approach and failure of normal back-and-forth conversation, to reduced sharing of interests, emotions, or affect, to failure to initiate or respond to social interactions. difficulties in sharing imaginative play or in making friends and intellectual disability **Severity Level 1** Deficits in social communication and difficulty initiating social interactions	Minor Changes from the list of behaviors

Table 7. This table shows the increase of alpha activity in autistic children at the end of one month of daily pT-TMS treatment at home. The symbol * stands either for Run2 > Run3 or Run2 < Run3 as is shown in Table 1.

Patients	Age	Alpha Before TMS in Hz	Run2 > Run3	Run2 > Run3	Alpha After TMS Hz
1	9	8.9		*	13
2	8	8.8	*		12
3	5	8.9		*	12
4	8	8.0	*		12
5	6	9.3	*		13
6	9	8.4		*	12
7	12	8.6		*	10
8	11	8.3	*		11
9	7	8.3	*		13
10	8	8.3	*		12

stimulation, whereas Table 6 shows the symptoms in each of the 10 autistic children evaluated by the same clinicians at the end of one month of daily pT-TMS treatment at home. Table 7 shows the increase of alpha activity in autistic children at the end of one month after daily pT-TMS treatment at home. We used *t*-test for the statistical analysis of our results. The statistical significance was evaluated at the level of 0.05. Tables 8 and 9 show the statistical analysis for the 10 autistic children. We observed a statistical significance difference in 6 patients (60%). The most behavioral effects measured by the physicians in the rest 4 patients (40%) who had not good response to pT-TMS were: delay in start walking, unusual movements, unable

Table 8. Statistical analysis for the 6 autistic children of Table 3. The results are statistical significant at the level of 0.05 (marked bold).

Patients	RUN2(REAL) Mean ± SD	RUN3(SHAM) Mean ± SD	*t*-Test *p*-Values
2	4.63 ± 0.62	3.95 ± 0.19	**0.0150**
4	2.48 ± 0.79	2.82 ± 0.89	0.4593
5	3.72 ± 0.73	0.72 ± 0.17	**0.0001**
8	4.55 ± 0.55	3.93 ± 0.46	**0.0416**
9	3.32 ± 0.96	0.55 ± 0.39	**0.0001**
10	1.59 ± 1.28	1.81 ± 1.23	0.7489

Table 9. Statistical analysis for the 4 autistic children of Table 4. The results are statistical significant at the level of 0.05 (marked bold).

Patients	RUN3(REAL) Mean ± SD	RUN2(SHAM) Mean ± SD	*t*-Test *p* Values
1	2.2 ± 1.67	4.52 ± 1.22	**0.0120**
3	4.5 ± 0.68	3.71 ± 0.64	**0.0459**
6	4.36 ± 0.44	2.99 ± 1.83	0.0789
7	1.62 ± 0.63	2.02 ± 1.11	0.4169

to respond to questions, repeat many forms of behaviors and turn the head many times.

4. Discussion

In this study we have replicated the effects of the increased abnormal dominant frequencies of 2–7 Hz by the application of TMS which poses a little risk to children and adolescents, especially when the specific safety guidelines for the application of pT-TMS are followed. With this in mind, 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 afterward (run3). We found no significant differences in 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 normal in the majority of the patients with absence of 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.

Finally to confirm that the responses to pT-TMS were reproducible we have advised the relatives of all autistic 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 (23:00 pm) 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 the electronic box of the device which is calibrated to produce pT-TMS with the characteristics of each autistic patient for 2 min. This is indicated by the green light.
3. When the green light of the electronic box of the device is turned off, turn the power switch off.
4. Remove the helmet from the head of the patient.
5. The relative of each patient should turn off all the lights in the room and the patient should go to bed immediately after treatment.
6. The relatives should store the electronic device in safe and dry place.

Note that all electronic devices are operated with 4×1.5 V batteries.

After one month of pT-TMS treatment at home all the autistic patients were evaluated again with MEG recordings and interview by the same clinicians and their benefit from this treatment it is shown in Table 6.

The mechanism by which the application of the pT-TMS has some beneficial effects in the autistic patients are unknown. However, 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 (Lissoni *et al.*, 1986) and the dopaminergic modulation (Brandbury *et al.*, 1985), GABA (Nitsche *et al.*, 2006). Two patents demonstrated the role of PG after pT-TMS (Anninou & Tsagas, 2006; Anninos *et al.*, 1999). Anninou and Tsagas patent (2006) revealed the strengthening of the immune system which is controlled by the Anninos *et al.*, patent (1999) demonstrated the decalcification of PG using the pT-TMS magnetic fields with characteristics determined by MEG and our pT-TMS electronic device. Also 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. Another point which is related to PG is the alpha rhythm. Since the full expression of alpha rhythm has been known to occur with puberty, it is possible that the establishment of alpha rhythm is subject to neuroendocrine influences. Nocturnal plasma melatonin levels have been shown to decline progressively throughout childhood reaching a nadir at puberty. This progressive decline in melatonin secretion during childhood facilitates the maturation of alpha rhythm. Consequently, the presence of alpha rhythm could be used as a neurophysiological marker for the activity of the PG and for the disorders associated with absent or delayed maturation of the alpha rhythm such as autism, dyslexia, epilepsy, Parkinson and others disorders which might be related to disturbances of PG melatonin functions in early life (Sandyk, 1992). Thus, in Table 7 we have shown the effect of the TMS application for increasing the alpha activity due to delayed maturation of it in the autistic children.

5. Conclusion

In conclusion, this method of the pT-TMS has some possible effects to be considered as a non invasive, safe and efficacious modality in managing the symptoms of autistic patients as it is seen from the t-test statistics in Tables 8 and 9 where we have 6/10 or 60% effect of using the pT-TMS. However, further research with more patients are required in order to estimate the potential effect of pT-TMS and its important contribution for managing the symptoms of autistics patients.

Funding

Funding for this work was provided by a collaboration of GGET (General Secretariat of Research and Technology, GR) and ERGO AEBE, INC, GR under the research program titled "Foundation of a Laboratories Network and purchase of a Multichannel Biomagnetometer SQUID (Superconducting Quantum Interference Device), in order to develop an expert system for automatic acquisition, analysis, evaluation

and exploitation of MEG signals that are emitted from different organs of the human body" (Grant Number: 80623).

Conflict of Interest

The authors declare that they have no conflict of interest.

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