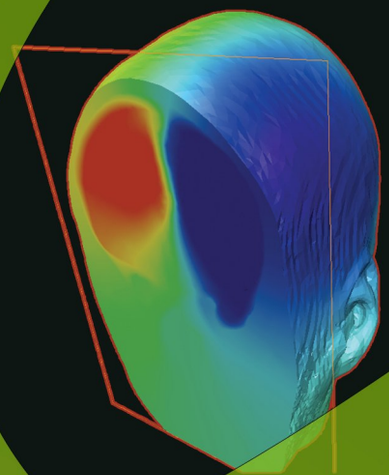

The SQUID as Diagnostic Tool in Medicine and its use with other Experimental Stimulation and Theoretical Methods for Evaluation and Treatment of Various Diseases

Photios A. Anninos

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Preface

In this book we are going to deal with an important subject namely, the magnetic fields emitted from human subjects for the purpose to understand and evaluate normal and abnormal functions and furthermore for the treatment of CNS disorders. The ionic currents which are originated from biochemical sources at the cellular level in the central nervous system (CNS) produce not only electric fields, but also magnetic fields.
These fields can be measured in the brain and surrounding tissues by very sensitive and sophisticated magnetic field detectors which are called SQUID's. The behavior of these fields can be predicted because they obey physical laws. The generation of the electroencephalogram (EEG) signals in the brain, in biophysical terms, is the exact way to determine the potential distribution at the scalp given a set of intracerebral current sources. In general terms, the field potential of a population of neurons equals the sum of the field potentials of the individual neurons. In order to understand the EEG phenomena, the activity of a population of neurons must always be considered. EEG phenomena can only be measured at a considerable distance from the source if the responsible neurons are regularly arranged and activated in a more or less synchronous way. Thus, while with the EEG it is very difficult to localize where a particular signal originates in the brain, in the case of the magnetic field and with the use of the (magnetoencephalogram) MEG it is easier to localize where the signals originate from the brain. The MEG is presently regarded as the most efficient method for recording the brain activity in real time for many reasons. Compared with the EEG, the MEG has unique sensitivity to the CNS disorders and normal functions of the brain. In addition, the MEG offers functional mapping information and measurement of brain activity in real time, unlike CT, MRI and fMRI which only provide structural, anatomical and metabolic information. With the MEG the brain is seen in 'action' rather than viewed as a still image. Another most important point is that the MEG has far more superior ability to resolve millisecond temporal activity associated with the processing of information which is the main task of our brain. Furthermore, another characteristic point is that the disturbing fields, namely the earth magnetic field and the urban magnetic noise (10^{-4} to 10^{-3} G) are almost constant over large distances, whereas the MEG falls off rapidly with distance. Other properties of the MEG that should be mentioned are the following. Neither electrodes nor a reference point are necessary for recording the MEG compared to the EEG; the transducers for the MEG need not touch the scalp, because the magnetic field does not disappear where conductivity is zero(free space).
The recordings of the MEG are the measurements of the magnetic fields perpendicular to the skull, which are caused by tangential current sources. By contrast, the EEG is a measure of both components. This means that the MEG measures the cortical activity lying in the sulci and not in the convexity of the gyri.
Thus, taking into account all of the above characteristic information, the magnetoencephalography is an important research field which is evolving quickly and a number of interesting findings in the following chapters we are going to be reported with respect to normal and abnormal functions of the human subjects.

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Summary

Since a flow of electrical charges produces a magnetic field, the current in the heart during depolarization and repolarization also produces a magnetic field which is about $5X10^{-11}T$ (tesla). To detect and measure such very weak magnetic fields it is necessary to use magnetically shielded room and very sensitive and sophisticated magnetic field detectors. Such sophisticated devices are the ones which are based on the Josephson effect of superconductivity (Ref) and are called SQUID's from the initials of the four words (Superconductive Quantum Interference Device). Such detectors operate at liquid helium temperature which is about 4K (-269^0C) and have the ability to detect magnetic fields of the order 10^{-15} T, whereas the magnetic field of the earth is $3X10^{-5}T$.

The recording of the heart's magnetic field is called magnetocardiogram (MCG), whereas the recording of the magnetic field produced by the flowing of ions in the brain is called magnetoencephalogram (MEG). The information provided by the MEG is entirely different from other imaging techniques and therefore shows considerable promise for brain studies as diagnostic tool and as such it is worth of discussing it in more detail.

Thus, while with the electroencephalogram (EEG) it is very difficult to localize where a particular signal originates in the brain, with the MEG and using different stimulation methods of external weak magnetic fields it is easier the location in the brain where the MEG signals originate from. Furthermore, using the above mentioned external weak magnetic stimulation and comparing the MEG records before and after the application of external magnetic stimulation (EMS) is shown a rapid attenuation of the high abnormal activity, characterized CNS disorders, followed by an increase of the low frequency components toward the patient's α-rhythm. Such an example we are given in a few sample chapters for the proposed book.

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Magnetoencephalography Evaluation of Febrile Seizures in Young Children

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Abstract. The aim of this study is to assess any cerebral dysfunction in young children, who experienced febrile seizures, by means of magnetoencephalography. Our study population included 15 children (9 boys, 6 girls) within the age range of 2 to 7 years. The magnetoencephalography data were recorded with a 122-channel biomagnetometer. Equivalent current dipoles were calculated for epileptic spikes on magnetoencephalography recordings according to the single dipole model. Of 15 children, 8 showed equivalent current dipoles that located at the left—temporal, right—temporal,occipital, and frontal lobe, as active regions responsible for febrile seizures. We assumed that the interictal epileptiform discharges are a consequence of febrile seizures. Of course, further study in a larger number of patients is needed to evaluate the exact role of the equivalent current dipoles, in young children, who experienced febrile seizures.

Keywords: magnetoencephalography; equivalent current dipole; febrile seizures; epileptiform discharges

1. Introduction

Febrile seizures are defined, by The International League Against Epilepsy, as ‘‘The seizures occurring in childhood after one month of age, associated with a febrile llness not caused by an infection of the central nervous system, without previous neonatal seizures or a previous unprovoked seizure, and not meeting criteria for other acute symptomatic seizures.[1] Magnetoencephalography has many advantages over electroencephalography (EEG) as it detects the magnetic fields originating from the intracellular currents within neurons, which are not attenuated or distorted by intervening tissues. Over the past several years, a whole-head system has been developed that enables its routine clinical application for epilepsy patients. Magnetoencephalography is indicated for localization of the irritative zone in lesional and nonlesional epilepsy surgery patients. Because of its high spatial and temporal resolution, magnetoencephalography provides promises to be a powerful tool in the investigation of normal and abnormal brain function including sensory, motor, memory, and cognitive processes in the developing brain in the future.[2-9] Analysis of brain function by functional magnetic resonance imaging (MRI) and positron emission tomography (PET) is based on the change of cerebral blood flow induced by neural activity whereas that of EEG and magnetoencephalography on the electric potential and magnetic changes induced by neural activity. Both EEG and magnetoencephalography are characterized by higher temporal resolutions than PET and functional MRI in measurements of brain activity. The major advantage of magnetoencephalography over EEG is that magnetoencephalography has higher localization accuracy. This is due to the fact that the different structures of the head influence the magnetic fields less than the volume current flow that causes the EEG. Magnetoencephalography provides a high spatial density of recording points, which is difficult to obtain with EEG. The magnetic fields are less distorted than electrical fields, because of the blurring effect of the skull, which acts as a low-pass filter for electrical potentials, providing, in this way, better conditions for the recording of fast activity, such as gamma-band oscillations. Moreover, inaccuracies in estimating the conductivities of the skull and other tissues of the head affect the interpretation of electrical much more than magnetic sources.[5-15] For all the above reasons, we preferred the use of magnetoencephalography for the evaluation of febrile seizures. Thus, in this pilot study, we investigated the possibility of any cerebral dysfunction caused by febrile seizures in young children, by means of magnetoencephalography,

due to the advantages of this method over the other imaging modalities.

2. Methods

Fifteen young children (9 boys, 6 girls) within the age range 2 to 7 years were referred to our lab by the pediatric department of our university general hospital to be examined with magnetoencephalography. There were no simultaneous EEG recordings. Informed consent for the methodology and the aim of the study was obtained from all the parents prior to the procedure. The magnetoencephalography recordings were carried out in a magnetically shielded room with a whole head 122-channel biomagnetometer (Neuromag-122; Neuromag, Helsinki, Finland).

All studies were performed precisely 10 days after fever subsided for the purpose of comparison. We analyzed the amplitude of magnetoencephalography spikes, at the magnetoencephalography sensor location, with the highest amplitude. We calculated the single ‘‘equivalent current-dipole’’ source, with a spherical model. We defined acceptable equivalent current dipoles as those with a goodness of fit to the model of >70% and with equivalent current dipole strength between 100 and 400 nAm. Normal participants of similar age who served as controls did not show equivalent current dipoles in their magnetoencephalograms.

3. Results

Table 1 exhibits the clinical characteristics of each child. After the magnetoencephalography signals were recorded, an equivalent current dipole model was estimated at each time point, within the encompassing signal segment, using a single dipole model. Of 15 children, 8 were shown equivalent current dipoles, which were located at the left—temporal (Figure 1, child no. 3), occipital (child no. 4), frontal (Figure 2, child no. 5); right—temporal (child no. 11), occipital (child no. 12), occipital (child no. 13), occipital (child no. 14), and occipital lobe (child no. 15), respectively, as active regions responsible for febrile seizures.

Table 1. The Clinical Characteristics of Each Child

No	Age (years)	Sex	No of events	Type of seizures	Duration of seizures	MEG	EEG	MRI/CT
1	2	M	1	GS	1-2 min	N	-	-
2	2	F	1	GS	1-2 min	N		
3	3	M	1	GS	1-2 min	ECD	-	-
4	4	M	1	GC	5 min	ECD	N	-
5	3	F	3	GS	1-2 min	ECD	-	CT: N
6	6	F	1	GS	1 min	N	-	-
7	6	M	1	G	1-2 min	N		
8	4	M	2	GSP	1-2 min	N	A	MRI: N
9	3	M	1	GS	1-2 min	N	-	-
10	5	M	1	GS	1-2 min	N	-	-
11	3	F	1	GS	1-2 min	ECD	-	-
12	3	F	1	GS	1-2 min	ECD	-	-
13	7	M	6	GS	1-2 min	ECD	N	MRI: N

14	5	F	2	GC	15 min	ECD	-	-
15	2	M	1	GS	1-2 min	ECD	-	-

Note: GS, Generalized tonic – clonic Simple febrile seizures; GC, Generalized tonic – clonic Complex febrile seizures; GSP, Generalized tonic – clonic Simple Partial seizures; A, abnormal; ECD, equivalent current dipole; F, female; M, male; MEG, magnetoencephalography; MRI, magnetic resonance imaging; N, normal; -, no exams.

Figure 1. The scalp ISO-field distribution and the equivalent current dipole (ECD) indicated by the arrow in the child no. 3 (Table 1). The coordinates are x (left/right), y (anterior/posterior), and z (superior/inferior).

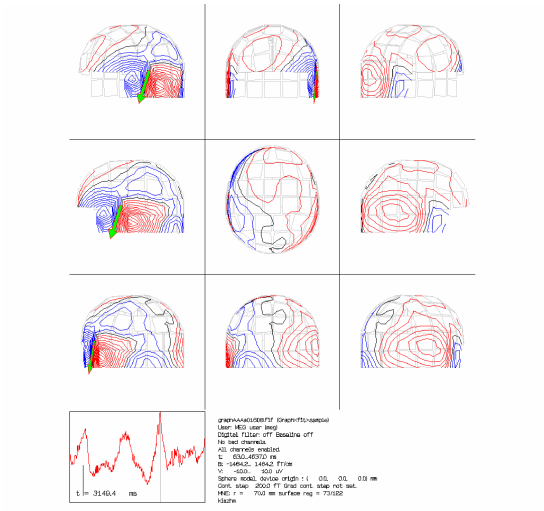
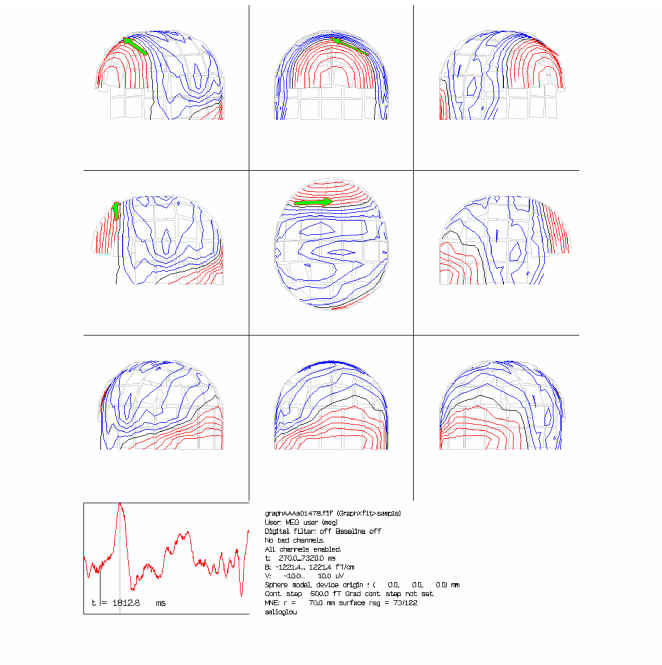


Figure 2. The scalp ISO-field distribution and the equivalent current dipole (ECD) indicated by the arrow in the child no. 5 (Table 1). The coordinates are x (left/right), y (anterior/posterior), and z (superior/inferior).



4. Conclusion

There is no unique neuronal generator that can explain a certain magnetoencephalography/EEG surface map. The
most simple and widely used model is equivalent current dipole, which assumes that the magnetic fields recorded at the surface can be accounted for by a unique dipolar source. This model arises logically from the physiological observation that the main neuronal sources of magnetoencephalography
and EEG activity consist of palisades of cortical pyramidal cells, with elongated apical dendrites oriented perpendicularly to the cortical surface. The estimation of a dipole model is reasonable only if the magnetic field on the surface has focal characteristics, and the number of possible sources can be anticipated with sufficient accuracy.5-15 We defined acceptable equivalent current dipoles as those with a goodness of fit to the model of >70%. Experimental studies proved the accuracy of equivalent current dipole localization of sources of interictal discharges. Studies introducing magnetoencephalography into the presurgical evaluation of epilepsy patients resulted in noninvasive detection of epileptic foci.[16,17] The magnetic activity of the brain is produced by cellular microcurrents, which emerge from ionic movements, due to the dynamical variations of the membrane potentials.
Although transmembrane, intracellular, and extracellular neuronal currents each produce surrounding magnetic flux, the neuromagnetic field recordable outside the head is a selective reflection

of intracellular currents flowing in the apical dendrites of pyramidal cells parallel to the skull surface. Magnetoencephalography is a technique for measuring the magnetic fields associated mainly with intracellular currents, while the EEG measures mainly extracellular field potentials. Intracellular currents are well modeled by the equivalent current dipole model. This model allows characterization of the source of neuronal activity in the brain and specifically its position and strength. This is particularly useful in focal epilepsies, in which small areas of brain tissue trigger the seizure and are
important in obtaining a good spatiotemporal localization of the ‘‘foci.’’ However, magnetoencephalography might be helpful in more complex epileptic patterns (generalized epilepsy) in characterizing the early start of interictal and ictal activity. In our study, 8 of 15 children were positive to equivalent current dipoles, whereas the rest were negative. This depends on the activity of the patients' brain during the magnetoencephalography measurements. If exhibits epileptic behavior, then we will observe equivalent current dipoles, otherwise we would not. From the 3 children (nos. 3, 4, 5) who experienced equivalent current dipoles, 1 had normal EEG and 1 normal CT. There was no clear relation with their clinical findings. Electroencephalography and neuroimaging studies are performed as dictated by clinical suspicion, and EEGs have been found to have limited value.18 Abnormalities on EEG do not predict the occurrence of future seizures or the subsequent development of epilepsy.[19,20] No evidence exists that epileptiform discharges in children with febrile seizures have any diagnostic or prognostic implications, even in the subgroup with complex febrile seizures. Neuroimaging is not necessary in children with simple febrile seizures.

Children who present complex febrile seizures and are neurologically normal are unlikely to have significant intracranial pathological conditions, such as a space occupying mass lesion, hemorrhage, hydrocephalus, abscess, or cerebral edema, that need emergency neurosurgical or medical intervention.[21] Three children (nos. 5, 8, 13) needed neuroimaging studies because we wanted to exclude structural damage that might cause seizures. Of the 3 children, 2 experienced multiple recurrences of febrile seizures, whereas the third experienced partial seizures (no. 8). Both types of febrile seizures must be detected by neuroimaging methods.[22-24]

Children with febrile seizures have a 6-fold excess (3%) of subsequent afebrile seizures and epilepsy than controls. The risk is 2% after a simple febrile seizure and 5% to 10% after a complex febrile seizure. Afebrile seizures usually start within 4 years. It is well known that EEG is an unhelpful diagnostic procedure, and we use it only when we want to distinguish ‘‘febrile convulsions’’ from convulsions with fever.[22-24] We tried to find a procedure that can provide us with prognostic indicators for possible epileptic behavior in children who experienced febrile seizures. We think that one of these prognostic indicators might be the existence of epileptiform discharges, which can be modeled by equivalent current dipoles. Of course, further research in larger number of patients is needed to evaluate the role of the equivalent current dipoles and the occurrence of epilepsy in young children who experienced febrile seizures.

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Multi-Channel MEG on Alzheimer Disease Patients

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*Abstract.*Magnetoencephalogram (MEG) recordings of 8 patients with advanced Alzheimer Disease (AD) and 9 normal individuals were obtained with a 122-channel whole head biomagnetometer SQUID (Superconductive Quantum Interference Device) to record the minute magnetic fields generated by the brain. The obtained MEG signals were analyzed using linear signal analysis techniques such as Fourier Transform in order to get the frequencies distribution of MEG values. The obtained frequencies from all MEG sensors located outside the scalp of each subject were stored for evaluation. From this evaluation it is seen that in patients with AD the dominant frequencies were significantly lower compared to normal individuals.

Keywords: Alzheimer patients; MEG; frequency; SQUID; brain.

1. Introduction

Alzheimer disease (AD) is the most common dementia in elderly people over 60 years of age. AD is a progressive disorder of memory, language, visuospatial function, and executive function associated with a high frequency of neurobehavioral abnormalities at some point in the course. The major pathological features of AD are brain atrophy with neuron loss, deficits in several neurotransmitter systems, neurofibrillary tangles, senile plaques and cerebrovascular amyloid [1].

The neuronal populations in the brain, emits spontaneous magnetic activity caused by ionic movements across the plasma membrane [2]. This activity, although exceedingly weak (it is about 10^{-8} of the earth's magnetic field which is equivalent to 50 μT), can be measured with a 122-channel whole head biomagnetometer SQUID (Superconductive Quantum Interference Device). This minute magnetic field is generated by simultaneous intra-cellular current from many neurons and it is called Magnetoencephalogram (MEG) [3].

The MEG recordings were carried out in a magnetically shielded room (Neuromag Ltd) [4, 5]. The pick-up-coils of the device are shaped like figure-of-eights to make them "near-sighted", i.e. sensitive to sources in the brain, but insensitive to the ambient noise fields. The device employs planar gradiometers, which record at each of the 61 measurement sites, the magnetic field component normal to the helmet-shaped dewar bottom surface.

During the recordings the subject was sitting in a chair with close eyes (in order to enhanced of the α-rhythm) and with his head covered by the helmet-shaped dewar. This way the subject is more stable and relaxed for MEG measurements [6]. A head position indicator, with three small coils, was fixed on the scalp. During MEG recordings the subjects were instructed to keep their eyes closed to avoid artefacts from eye flickering.

Although the importance of MEG recordings in the investigation of normal and pathological conditions of the brain (and especially in the study of epileptic phenomena and Parkinson disease), has been noticed by several authors [7-10], this methodology was also applied on Alzheimer's disease (AD) patients [11-18]. The main difference with the above stated articles is that each of them uses different approach compared to ours in which it was used the dominant frequency obtained from the power spectrum after Fourier statistical analysis.

2. Methods and materials

2.1. Subjects

AD patients were referred to the Laboratory of Medical Physics, by practicing neurologists. All patients had been diagnosed to suffer from advanced stage AD (estimated abbreviated mental test score range 1-2). This test is exclusively based on question – response evaluation of patient cognitive ability [19]. MEG recordings were obtained from 8 patients with AD and from 9 normal subjects (all subjects were women). The age of the AD patients ranged from 57 to 83 (mean=68.4, SD=8.3), while the normal subjects ranged from 48 to 80 (mean=65.3, SD=9.2). This difference was not statistically significant (p=0.19).

In all cases informed consent for the methodology and the aim of the study was obtained from all normal subjects and from all patients relatives prior to the procedure.

2.2. Data acquisition, analysis

From the brain of each subject were obtained 122 MEG records. Each MEG signal was recorded for 32 consecutive epochs. Each epoch was 1 sec duration, and was digitized with a sampling frequency of 256 Hz (frequency resolution of the power spectrum being 1 Hz). The associated Nyquist frequency to this sampling rate was 128 Hz, which was well above of constituent frequency component of interest in our MEG recordings and avoiding aliasing artefacts. The MEG data were stored in a PC peripheral memory for off-line statistical analysis. The MEG signal was band-pass filter with cut-off frequencies of 0.1 and 60 Hz. This method, by its nature (i.e., temporal and spatial averaging), eliminates short-term abnormal artifacts in any cortical area, while it retains long lasting localized activation phenomena. The MEG data of the 122 MEG sensors outside the scalp were analyzed using Fourier statistical analysis [6]. In this Fourier statistical analysis each MEG record it was stored for off-line statistical analysis in which it was trying to find the best fit of one trigonometric mathematical function [20] as it is seen in the following equation:

$$y(t) = a_0 + \sum_{K=1}^{N} (a_k \cos K\omega_0 t + b_k \sin K\omega_0 t) \qquad (1)$$

where the coefficients a_k, b_k are given in equations (2) and (3) respectively, whereas the constant a_0 is given by equation (4):

$$a_k = \frac{2}{T}\int_0^T s(t)\cos K\omega_0 t dt \qquad (2)$$

$$b_k = \frac{2}{T}\int_0^T s(t)\sin K\omega_0 t dt \qquad (3)$$

$$a_0 = \frac{1}{T}\int_0^T s(t) dt \qquad (4)$$

(In the above equations 1, 2, 3 and 4, K takes values from 1 up to N, where N is the highest harmonic, ω_0 is the angular frequency in Hz which is defined as: $\omega_0 = 2\pi/T$, where T is the period in seconds).

Finally, the function s(t) is the signal which is recorded with the SQUID sensors.

In this analysis were calculated, for each of the 122 MEG measured points, the average amplitude distribution of the spectra densities from the 32 power MEG spectra obtained as was stated above.

With the average spectra it was calculated the dominant frequency amplitude from each MEG sensor located outside the scalp of the subject. An example of the MEG data recordings for 10 seconds duration is given in Fig.1

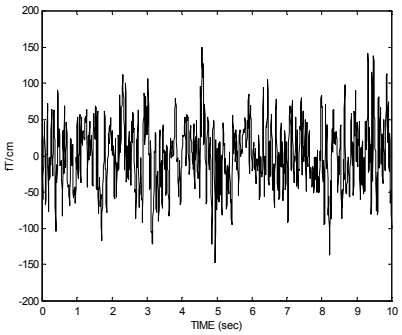

Fig. 1. This figure shows the MEG raw data from one of the 122 channels obtained from one randomly selected AD patient.

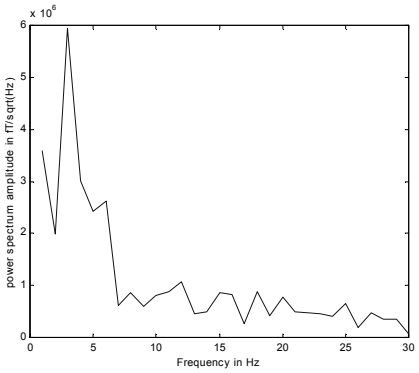

Fig. 2. The power spectrum of Fig. 1 MEG raw data in which it is shown the dominant frequency of the first maximum of the power amplitude spectrum.

In addition in Fig. 2 is given the power spectrum of the raw data of Fig. 1 in which it is seen the dominant frequency namely the first power maximum amplitude of a certain frequency. Similar exhaustive power spectra for frequency analysis was done by Fernandez et al., [18].

2.3. Statistical analysis

Statistical analysis and graphs were performed using the GraphPad Prism® 4.0 package (San Diego California USA, www.graphpad.com). The unpaired two-tailed t-test was used for testing relationships between groups of continuous

variables. The continuous variables refer to the group of values obtained with the SQUID from the Alzheimer patients and which were compared to another group of values of normal subjects. These values were obtained after Fourier statistical analysis. These continuous variables are the dominant frequencies in both groups. All p-values are two sided and p-values <0.05 were used for significance.

3. Results

The Fig. 3 shows the spatial statistical distribution for the dominant frequencies of each MEG sensor located outside the scalp obtained using MEG measurements from AD and normal subjects.

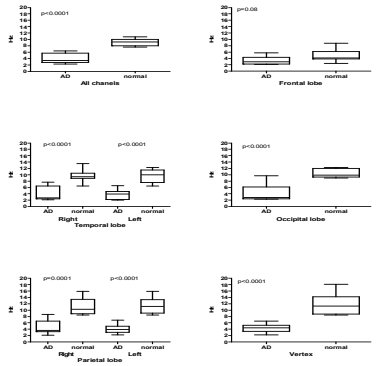

Fig. 3. The spatial statistical distribution for the dominant frequencies of each brain area of the emitted magnetic activity obtained using MEG measurements from AD and normal subjects.

These sensors were grouped from the constructor in such a manner so that to be in accordance with the brain regions (Fig. 4).

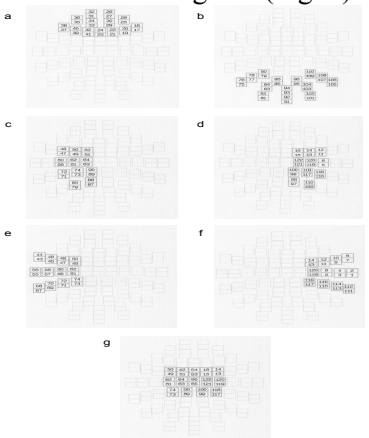

Fig. 4. The different locations of the group of the sensors in the SQUID with respect to corresponding brain regions. The figures 4a, 4b, 4c, 4d, 4e, 4f, 4g corresponds to the frontal, occipital, left parietal, right parietal, left temporal, right temporal lobe and vertex respectively.

The Neuromag-122 system inherently uses device coordinate system. The recorded signals represent field components at fixed sensor locations in the device coordinate system. The origin of this coordinate system is located at the center of the posterior spherical part of the helmet with y-axis pointing from back to front, x-axis from left to right and z-axis pointing up. Thus the position of subject's head in respect to the measurement probe does not affect the way the signals are recorded.

As it was discussed in the methods (2.3.) by applying the unpaired two-tailed t-test between normal and AD patients we found that the difference is highly significant for all areas assessed. It is evident that in most of the cases the range of the frequency values noted in patients is far lower than the minimum value obtained in healthy controls.

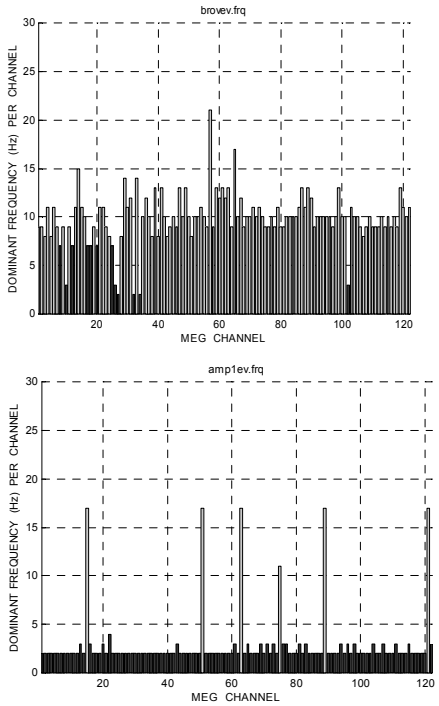

Fig. 5. (a). The dominant frequency amplitudes from the 122 channels which correspond to the 122 brain points of 1 AD patient. The code of the name (for easy identification) of this patient is given in the top of the graph.

(b). The dominant frequency amplitudes from the 122 channels which correspond to the 122 brain points of 1 normal individual. The code of the name (for easy identification) of this normal subject is given in the top of the graph.

Furthermore, Fig. 5(a) and 5(b) shows the distribution of dominant frequencies in all 122 channels for one AD and one normal subject correspondingly. Noted that all frequencies were sharply decreased in the AD patient in almost all channels analyzed.

4. Discussion and conclusions

With the 122 channel SQUID it is possible to record MEG signals which correspond to the 122 brain points in a very short time of about 32 sec. These MEG signals, which were obtained from the AD patients, are analyzed using Fourier statistical analysis from which it was obtained the dominant frequencies distribution. These frequencies correspond to the first dominant amplitude of the MEG Fourier power spectrum obtained from each of the 122 measured brain points.

In the above AD patients it is observed that these MEG signals are characterized by low frequencies in the range of 2-7 Hz and absence or very low α-rhythm (in the range 8-13Hz). The above obtained results are statistically significant.

With respect to the above observed, decreased frequencies in the AD patients it maybe possible to conclude that is due to the reduction of acetylcholine which is a neurotransmitter substance associated with memory processes. It is also due to the diminished levels of norepinephrine, somatostatin and dopamine which all are controlled by the pineal gland which also controls the neural activity of the brain. All these substances play an important role for the normal brain function in all subjects [21, 22].

In addition, the observed increased activity in low frequencies and also the discrepancy between the EEG and the MEG in this paper which also was observed in Fernandez et al., [18] are due to the use of a reference recording point system, whereas in the MEG which combines high temporal and high spatial resolution is independent of any reference recording system. Furthermore, the increase in the activity of low frequencies power values especially for the delta frequency band is correlated with the cognitive and functional status of the AD patients as have been stated also by other authors [15-18].

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The Application of External Magnetic Stimulation for the Treatment of Parkinson Diseased Patients

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*Abstract.*The aim of this study was to investigate the influence of external transcranial magnetic stimulation (TMS) in parkinson 's diseased (PD) patients using a whole-head 122-channel magnetometer and Fourier statistical analysis. The examined group consisted of 20 patients (12 males and 8 females; mean age 65 years: range 49-80 years). External transcranial magnetic stimulation in the order of pico Tesla (TMS) was applied on the above patients with proper field characteristics, which were obtained prior to TMS (magnetic field amplitude: 1-7.5pT, frequency: the α-rhythm of the patient: 8-13 Hz). The MEG recordings after the application of TMS shown a rapid attenuation of the high abnormal activity followed by an increase of the α-rhythm (8-13 Hz). The patients' responses to the TMS were a feeling of relaxation and partial or complete disappearance of tremor, muscular ache and levodopa induced dyskinesias as well as rapid reversed visuospatial impairment, which were followed by a corresponding improvement and normalization of the MEGs.

Keywords: 122-channel magnetometer, Parkinson's disease, external magnetic stimulation

1. Introduction

Clinical applications of transcranial magnetic stimulation (TMS) was first reported by Baker et al. (1) and has been widely used to assess possible changes secondary to PD. The use of single- and aired-pulse TMS, two varieties of the original technique, disclose multiple functional alterations of the corticospinal pathway (2). The use of TMS in PD investigations began about 10 years ago. Then it had become clear that TMS could provide information not only on the conductivity of corticospinal neurons, but also on other properties of the primary motor cortex, such as excitability (3). In turn, basic evidence strongly suggested that excitability was under the influence of multiple afferences to the motor cortex itself, among which those arising from the basal ganglia (4). Hence, a new insight arose into the pathophysiology of PD as well as of other movement disorders. TMS has provided substantial new pathophysiological insights, which point to a central role of the primary motor cortex in the movement disorder typical of PD. Recently several clinical trials have suggested the therapeutic efficacy of repetitive TMS (rTMS) in patients with PD (5-10).The goal of this study is to report the beneficial effects of external TMS (in the order of pico Tesla), on PD patients using MEG measurements and statistical analytic techniques in the frequency domain.

2. Materials and Methods

Twenty PD patients (12 males, 8 females; range 49-80 years) were referred to our laboratory by practicing neurologists. All patients had diagnosed independently to suffer from idiopathic PD with no history of other neurological disease. Patients had normal routine serum biochemical studies. Informed consent for the methodology and the aim of the study was obtained from all patients prior to the procedure. All patients were initially placed on levedopa/carbidopa (Sinemet 25/250)(1 tablet twice daily), but due to progressive deterioration in their motor disability the dosage was increased to 3 ½ tablets/day (1/2 tablet every 2 hours). They remained on this dosage for more than 4 years. Biomagnetic measurements were performed using a whole-head Neuromag 122 MEG system in a magnetically shielded room. The time taken for each recording was between 1-2 min. Afterwards, external transcranial magnetic stimulation in the order of pico Tesla (TMS) was applied with proper field characteristics, which were obtained prior to TMS using an electronic device (magnetic intensity: 1-7.5 pT; frequency: the α-rhythm of the patient: 8-13 Hz) (**11,12**). The coils of this device were placed on the patient's scalp and weak magnetic fields, were applied for total 6 minutes (2 minutes over each of the following areas: left and right temporal regions, frontal and occipital regions, and over the vertex). This device consists

of a generator that produces square waves of low frequencies magnetic field in the range from 2-13 Hz to a group of coils of 1cm diameter. The coils are enclosed between two parallel plane surfaces in such a way that their axis is situated perpendicular to these surfaces. The time between the first MEG and the MEG obtained after the application of the TMS was about an hour. To confirm that the responses to TMS were reproducible, the patients were instructed to apply TMS with the same characteristics nightly at home. Since this resulted in the same reaction to the one obtained in our laboratory and since this effect was sustained for a period more than a month, we preliminarily concluded that the application of the TMS is a non-invasive, safe and efficacious modality in management of PD patients.

3. Results

Table I shows each patient's clinical report and their response to TMS. All the patients have diagnosed to suffer from idiopathic tremor, rigidity, and dyskinesia PD on the basis of clinical observations and routine EEG recordings. The patients were divided into two subgroups according to the degree of their responsiveness to TMS. The first subgroup included patients who exhibited only partial response (PR) to TMS (i.e., their tremor or muscular ache or dyskinesias recurred within 12 months after TMS and partial appearance of α-rhythm with low amplitude in their EEG). The second subgroup included patients who demonstrated a favorable response (FR) to TMS (i.e., they were free from the above symptoms for at least one year after TMS and the appearance of α-rhythm with high amplitudes in their EEG) (table I). Table II shows that 6 patients (30%) were classified as partial responders (PR) and the remaining 14 (70%) exhibited a favorable response (FR) to TMS. From the partial responders to TMS, normal EEG (i.e., the appearance of high amplitude of power spectrum in the α- rhythm frequency) was seen only in 1 patient (16.67%). In contrast, 12 out of 14 patients (83.84%) who showed a favorable response to TMS had normal EEG (i.e., the appearance of very high amplitude power spectrum in the α-rhythm frequency). This difference was statistically significant ($p<0.001$,chisquare=8.80). At this point it should be mentioned that the EEG and the MEG diagnosis before and after TMS was based on the appearance of α-rhythm amplitude in their power spectra amplitude distribution.

4. Discussion

The improvements in the present study could be attributed largely to dopamine release. This is supported by an experimental study in which repetitive TMS (rTMS) lead to increased release of dopamine in the striatum and frontal cortex (**13**). Strafella et al. (**8**) showed that rTMS of the prefrontal cortex induces the release of endogenous dopamine in the ipsilateral caudate nucleus as detected by positron emission tomography in healthy human subjects. The rTMS-induced release of dopamine in the caudate nucleus could be a consequence of direct stimulation of the corticostriatal axons (**14**). GABA is the dominant inhibitory neurotransmitter of the motor cortex. Berardelli et al. (**6**) recorded an increase in the duration of the TMS-evoked SP during a 20-pulse train of suprathreshold rTMS in healthy volunteers as well as in PD patients. Mally and Stone (**15**) have reported sustained improvements in movement-related measures with various regiments of repeated TMS pulses administered with round coils over periods of weeks to months. Siebner et al. (**7**) recorded an increase in the duration of the TMS-evoked SP in PD after 15 trains of 5-Hz rTMS over the hand area. This means that 5-Hz rTMS is capable of inducing short-term change in the excitability of intracortical inhibitory circuitry in PD patients. As dopamenergic drugs result in a similar modulation of the SP, the facilitatory effect of 5-Hz rTMS on intracortical inhibition might be a candidate mechanism that mediates the beneficial effect of 5-Hz rTMS of primary motor area in PD patients.

In this study the patients' responses to the TMS were a feeling of relaxation and partial or complete disappearance of muscular ache and levodopa-induced dyskinesias as well as rapid reversed of visuospatial impairment. This clinical improvement was followed by a corresponding improvement and normalization of the MEGs recorded after the application of TMS. Assuming that the MEG of PD patients is a reflection of the pathogenesis in the substantia nigra, dopaminergic functions and sympathetic ganglia, it appears that the application of the TMS has an immediate and beneficial effect on the dynamical condition of these pathological neural structures.

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Table I. Individual clinical data for each PD patient (N=20)

SUBJECTS	*AGE*	*AGE START*	*EEG DIAGBMS*	*EEG DIAGAMS*	*MEG DIAGBMS*	*MEG DIAGAMS*	*IMPROVEMENT (YEARS)*
MEN	77	55	P	N	A	A	2
	61	52	P	N	A	N	2
	79	58	N	N	A	N	3
	57	63	P	N	A	A	3
	69	57	P	N	A	A	3
	71	52	P	N	A	N	3
	49	45	P	N	A	N	2
	55	48	P	N	A	N	2
	67	63	P	P	A	N	3
	66	61	N	N	A	N	2
	58	50	P	N	A	N	3
	80	64	P	N	A	N	2
WOMEN	58	47	P	N	A	N	2
	72	67	P	N	A	N	3
	62	55	A	N	A	N	2
	76	61	P	N	A	N	2
	58	50	P	P	A	N	3
	52	50	P	N	A	A	2
	68	58	A	P	A	A	2
	65	49	P	N	N	N	2

A: abnormal; P: partial normal; N: normal diagnosis; DIAGBMS: diagnosis before TMS; DIAGAMS: diagnosis after TMS

Table II. Classification of the examined PD patients according to their EEG and MEG diagnosis and their response to magnetic stimulation. The results were of statistical significance ($p<0.001$ chisquare=8.80)

Response	*NORMAL EEG*	*ABNORMAL EEG*	*TOTAL*
PR	1	5	6
FR	12	2	14
TOTAL	13	7	20

The Chaos Theory

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Recently it has be given a great deal of attention in getting informations from experimental time series .The basic idea for these systems is the study of the concept of the strange attractor.
With this we mean the dynamics of the system is such that so to be able to move in a well defined organized region of the phase space without to go to a limited cycle or to a limited point. One of the most important dynamical parameter to describe the system is the correlation dimension D and the Lyapunov exponent λ.
The correlation dimension gives the dimension of the phase space or the 2D+1 number of differential equations which we need for the description of the dynamics of the system. In order to determine the correlation dimension D we use the method of Grassberger-Proccacia which is based in the theorem of reconstruction of the phase space given by Takens.
According to the Takens theorem the dynamics of the system under consideration can be substitute experimentally from one only observed dynamical component of a time series as is in our case the MEG. So for the discrete time series
$B_i=B(t_i)$ (i=1,2,3… N) of the MEG the vector construction V_i will be given by the following equation:

$$V_i = (B_i, B_{i+\tau}, \ldots B_{i+(m-1)\tau}) \quad (1)$$

So the phase space construction is done with the use of the above vectors.
If the phase space is m-dimension then the vectors that should be constructed will be m-dimension also.
Therefore in our MEG experimental time series of length N i.e $B_1, B_2, B_3 \ldots B_N$
the vectors which will be constructed will be:

$$\begin{aligned} V_i &= (B_1, B_{1+\tau}, \ldots B_{1+(m-1)\tau}) \\ V_i &= (B_2, B_{2+\tau}, \ldots B_{2+(m-1)\tau}) \\ &\vdots \\ V_i &= (B_N, B_{N+\tau}, \ldots B_{N+(m-1)\tau}) \end{aligned} \quad (2)$$

The time τ in the above equations is an appropriate time delay constant chosen arbitrarily (usually is equal to the first crossing of the autocorrelation signal with time). The vectors which will be constructed with the above assumptions will be:
$n=N-(m-1)\tau$
If we consider for simplicity the embedding dimension to be m=2 then for a given time τ we will have the following vectors:

$$\begin{aligned} V_i &= (B_1, B_{1+\tau}) \\ V_i &= (B_2, B_{2+\tau}) \\ &\vdots \\ V_n &= (B_n, B_{n+\tau}) \end{aligned} \quad (3)$$

where $n=N-(m-1)\tau=N-(2-1)\tau=N-\tau$
Using the method of Grassberger-Proccacia we can calculate for every vector combination the Euclidian distance namely:

$$\begin{aligned} V_1 - V_2 &= ((B_1 - B_2)^2 + (B_{1+\tau} - B_{2+\tau})^2)^{1/2} \\ V_1 - V_3 &= ((B_1 - B_3)^2 + (B_{1+\tau} - B_{3+\tau})^2)^{1/2} \\ V_1 - V_4 &= ((B_1 - B_4)^2 + (B_{1+\tau} - B_{4+\tau})^2)^{1/2} \\ &\vdots \end{aligned} \quad (4)$$

If the dynamics of the physical system is chaotic the evolution of the system in the phase space goes to a fractal, which it is called strange attractor. If there is a strange attractor this can be described by a geometrical parameter which is known as the fractal correlation dimension D.
According to the method proposed by Grassberger-Proccacia the correlation dimension D can be calculated from the experimental time series MEG with the following correlation integrals C(r,m) which are given by:

$$C(r,m) = \lim_{n\to\infty} (n(n-1)/2)^{-1} \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \Theta(r - |Vi - Vj|) \quad (5)$$

where Θ is the Heaviside function which is defined as:

if $\Theta(r - |Vi - Vj|) > 0$ then we get 1 (6)

if $\Theta(r - |Vi - Vj|) < 0$ then we get 0

In the above equation m is the embedding dimension and n is the number of vectors which are constructed for the time series with N elements given by $n=N-(m-1)\tau$.

The correlation integrals C(r,m) give the space relation of the points of the strange attractor and is calculated from the different values of r in the region from zero up to the r_{max} where the r_{max} is the maximum possible distance of the chosen time series and is defined as $r_{max} = (m)^{1/2}(B_{max}-B_{min})$.

In this relation the B_{max} and B_{min} are the max and min values of the time series.

The comparison is done for all possible combinations of vectors and all possible values from r=0 to $r=r_{max}$ and for all possible values of m. For a given dimension m comparison is done with all possible combinations for one sphere of radius $st=r_{max}/k$ (where k is an arbitrary number which it can be selected in order to divide the sphere radius r_{max}) of r and $|V_i - V_j|$ vectors.

If the $st > |V_i - V_j|$ then according to what we have said before the result from the equations (5) and (6) will be 1 and then will search for another combination of vectors and if we have the same result we will put again 1 in the previous result. Thus we will continue for all possible combinations of $|V_i - V_j|$. Then we get the sphere of radius 2st and compare this with all possible vectors $|V_i - V_j|$ where the i,j varying according to the equation (5). Then we add all the ones(1) of the radius st,2st,3st…r_{max}. If the sum for all ones(1) for every internal sphere is divided with the factor n(n-1)/2 then we get the correlation integral C(r,m) for the sphere.

The correlation integral is found to behave for every r as:

$C(r,m) \sim r^D$ (7)

Therefore the correlation dimension D in the phase space will be:

$\ln C(r,m) = D \ln r$ (8)

and

$$D = \lim_{\substack{r \to 0 \\ m \to \infty}} (\partial \ln C(r,m) / \partial (\ln r)) \quad (9)$$

In order to see if the signal of the time series is chaotic or not we have to examine the slopes of equation (9) with respect to lnr.

If there is one value which remains constant even though the signal is embedded consequently in phase spaces of higher dimensions these slope values give the correlation dimension of the strange attractor for one non-chaotic signal, whereas if there is not such constant value the signal is chaotic.

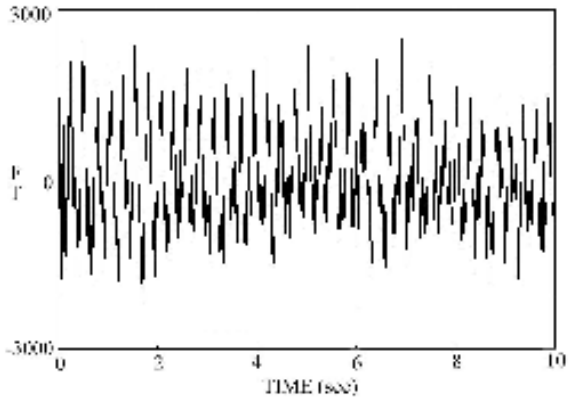

Figure 1.The time series of 10sec duration of an MEG which was taken from a brain point of a Parkinson patient before pTMS.

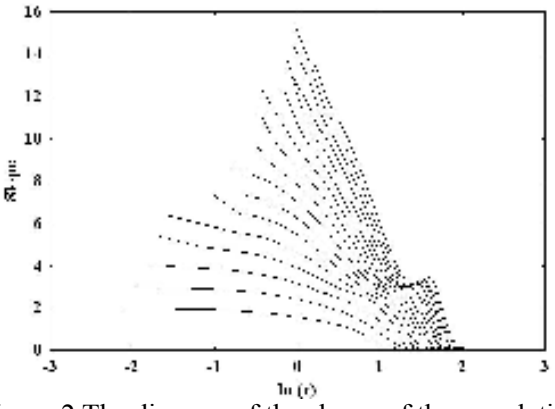

Figure 2.The diagram of the slopes of the correlation integrals as a function of lnr for the MEG of figure 1. In this diagram we can see the correlation dimension to be about 3.1

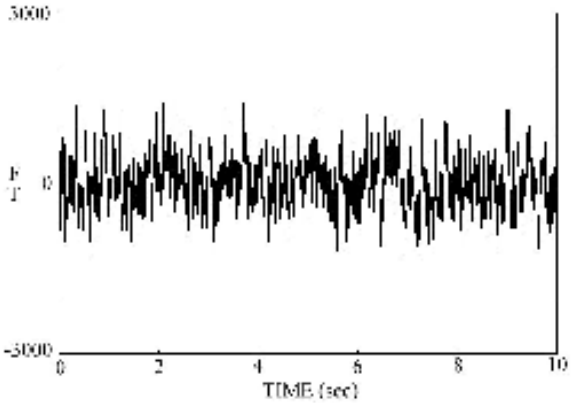

Figure 3.The MEG of 10sec duration from the same brain point
of figure 1 after pTMS.

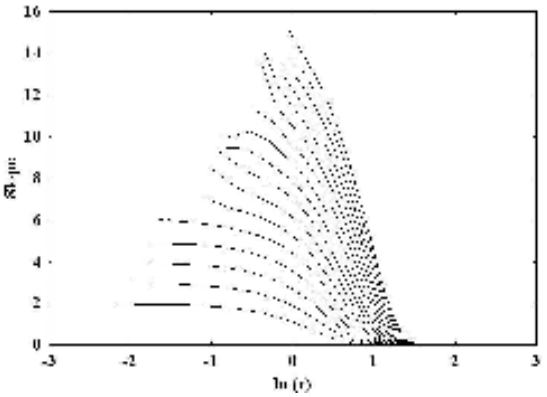

Figure 4. The slopes of correlation integrals as a function of the lnr for Figure 3.

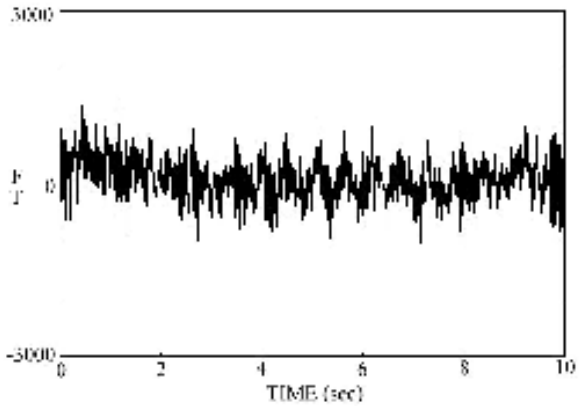

Figure 5.The MEG of 10sec duration for a normal subject.

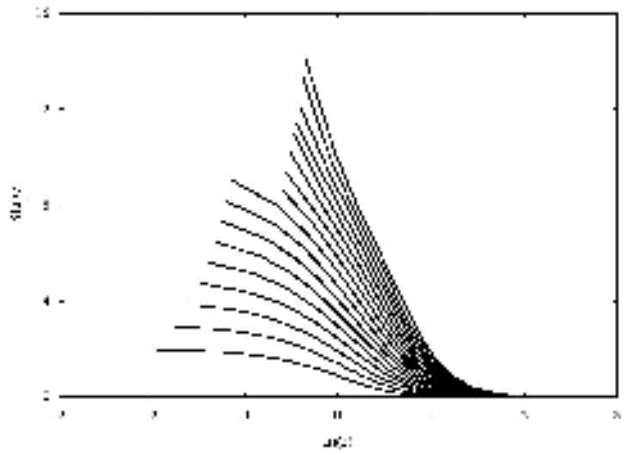

Figure 6. The slopes of the correlation integrals as a function of lnr for figure 5.

Another point in the non-linear analysis of time series is the evaluation of the Lyapunov exponents as a function of the evolution time. This idea was proposed by Wolf.
The Lyapunov exponents gives us a way to measure the chaotic behavior of a system by describing the variation of the deviation from the neighbor tracks. In most of the cases we need to measure the largest Lyapunov exponent, by examining the evolution of one small displacement of a vector ξ_0 in a given point of the strange attractor. For a chaotic system the vector ξ
Increases exponentially according to the following equation

$$\xi_t=\xi_0 e^{\lambda t} \qquad (10)$$

where $\lambda>0$

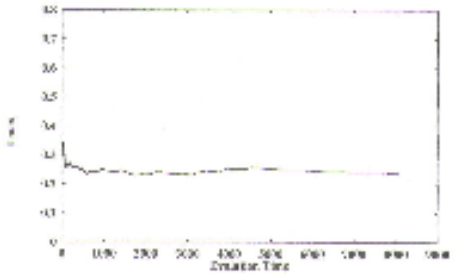

Figure 7. The Lyapunov exponent for a chaotic system.

Nonlinear Analysis of Brain Magnetoencephalographic Activity in Alzheimer Disease Patients

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Abstract. Objectives: Non-linear analysis was applied on MEG signal of Alzheimer Disease (AD) patients in order to investigate the underlying complexity of the brain dynamics.*Materials & methods:* A Single channel SQUID was used to record the MEG signals in 9 AD patients and 5 normal individuals. The magnetic activity, for each patient, was recorded from a total of 64 points of the skull (32 points from each temporal lobe). Non linear analysis was applied in the abnormal MEG points of the brain.*Results:* In AD patients some recorded points were found with high amplitudes and low frequencies in magnetic activity. By applying non-linear analysis in these records were found low values in the correlation dimension D of the reconstructed phase space. *Conclusions:* SQUID obtained MEG signaling from brains of AD patients showed a lower complexity compared to the brain of normal subjects.

Key Words: Alzheimer Disease ; chaos ; MEG

1. Introduction

Usingagnetoencephalographic (MEG) measurements we recorded the brain activity from patients who were suffering from Alzheimer disease (AD). In these recordings we used the biomagnetometer SQUID (Superconductive Quantum Interference Device) which can detect the magnetic fields emitted from the brain. The magnetic activity of the brain is produced by cellular micro-currents, which emerge from ionic movements, due to the dynamical variations of the neural membrane potentials. Such magnetic fields emitted from the brain are very weak (of the order of pT=10^{-12} T) and only SQUID can detect and record these fields (SQUID has the ability to detect magnetic fields of the order of 10^{-15} T (=1fT)).

Some clinical, as well as, theoretical studies, which have been published in the previous decade, have shown that the MEG method presents a number of very important and crucial advantages compared to the EEG (electroencephalogram) method (1-4). More recent studies (5-12), have proved the equivalent accuracy of the two techniques and that they are complementary.

Although the importance of MEG recordings in the investigation of normal and pathological conditions of the brain (and especially in the study of epileptic phenomena), has been noticed by several authors (3, 4, 13-16), this methodology was also applied on Alzheimer's disease (AD) patients (17-20). We detected abnormal brain magnetic activity, which exhibited high amplitudes and rhythmicity.

According to the theory of nonlinear dynamical systems and chaos (21, 22) the dynamics of any physical or biological system can be quantified and described by means of some new terms and concepts, such as the strange or chaotic attractor, the correlation dimension of the reconstructed phase space, the Lyapunov exponents and so on. These concepts reflect some geometrical properties of the reconstructed phase space of the dynamical system under consideration and it can be extracted. Of vital importance in the chaotic analysis of a dynamical system is the evidence for the existence of low dimension chaotic attractors and the estimation of the correlation dimension D of the attractor. In the present work the MEG time-series of the cortical magnetic activity of patients suffering from AD were recorded. In order to investigate for the existence of low dimensional strange attractors and to estimate the corresponding correlation dimension D the Grassberger-Procaccia algorithm (21, 22) was applied on the experimental time-series.

2.Material & methods

2.1. Patients and Recordings

AD patients were referred to the Laboratory of Medical Physics in Alexandroupolis, Greece, by practising neurologists. All patients had been diagnosed by the referral neurologists independently to suffer from Alzheimer disease. The age of patients ranged from 55 to 72 years (mean=65.2, SD=6.3). Furthermore, the clinical symptomatology of AD patients included moderate memory disturbances, speaking and communication difficulties and orientation disorders (estimated abbreviated mental test score range 3-5). Whereas, all the control population are free of the above mention clinical symptomatology. The onset of symptomatology of AD patients ranged from 1 to 4 years before examination. In all cases informed consent for the methodology and the aim of the study was obtained from all patients prior to the procedure. The SQUID examination of patients has been approved by the local hospital authorities.

Biomagnetic measurements were performed using a second order gradiometer SQUID model 601 of the Biomagnetic Technologies Inc., which was located in an electrically shielded room. The noise level of the environment was of the order of 50 fT/√Hz. During the recording procedure the patients were relaxed lying on a wooden bed, with closed eyes, in order to avoid artefacts from eye flickering. The MEG recordings were performed after positioning the SQUID sensor 3 mm above the scalp of the patient, with the use of an optic positioning system.

The MEG measurements consisted of data recorded from the scalp of each patient at specified points as defined by a recording reference system. This reference system is based on the International 10-20 Electrode Placement System (23) which uses any one of the standard EEG recording positions as its origin (3). In this study we used the P3, P4, T3, T4, F3 and F4 recording positions. The reference system was devised to retrieve maximal information from a specified area of the skull given that the gradiometer coil is theoretically equally sensitive to all magnetic flux lines perpendicular to a circular area of the brain. In our case, this circle has an effective diameter of 2.36 cm, i.e., the diameter of the SQUID sensor coil. Around the origin (T3 or T4 for temporal lobes) a rectangular 32-point matrix was used (4 rows x 8 columns, equidistantly spaced in a 4.5 cm x 10.5 cm rectangle) for positioning of the SQUID (Fig.1).

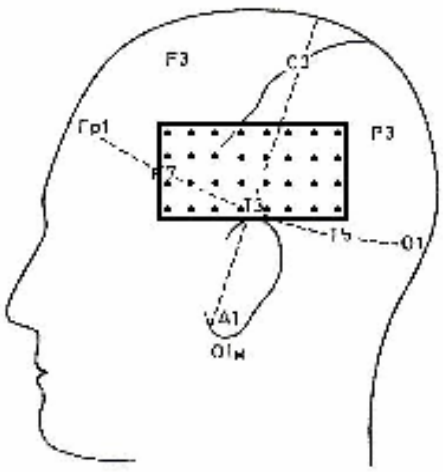

Figure 1
This figure is showing the 10-20 International Point System the points of which are served as origin in our rectangular reference system.

The MEG was recorded from each cerebral hemisphere at each of the 32 matrix points on the scalp for 32 consecutive epochs. Each epoch was of 1 sec duration and was digitised with a sampling frequency of 256 Hz (frequency resolution of the power spectrum being 1 Hz). The MEG signal was band-pass filtered with cut-off frequencies of 0.1 and 60 Hz. The MEG recordings were digitized at 256 Hz using a 12 bit precision analog to digital converter and were stored in memory for off-line Fourier statistical analysis, and averaged amplitude spectra were calculated for each sampling position.

2.2. Data analysis

We applied nonlinear analysis to the MEG recorded from the AD patients. The nonlinear analysis is a powerful technique for the estimation of the dimension of the strange attractor which characterizes the MEG time series obtained from normal and AD patients. For the estimation of the dimension of the strange attractor we have considered the method proposed by Grassberger and Procaccia (22) which is based on the Theorem of the reconstruction of the phase space introduced by Takens (24).

Then, according to their method, the

dynamics of the system under consideration can experimentally reconstructed from the observed time series of a single observable dynamic component, as it is in our case MEG. Thus, for the discrete time series $B_i=B(t_i)$ (i=1,2...N) of the MEG, which is measured experimentally by the SQUID, the vector construction of V_i is given by the following equation:

$$V_i=\{B_i,B_{i+\tau},...,B_{i+(m-1)\tau}\} \quad (1)$$

This equation gives a smooth embedding of the dynamics in a m-dimensional phase space, and the resulting phase trajectory in the phase space, is topological equivalent to the original phase space. The reconstruction time τ, is a suitable delay parameter, which may be chosen arbitrary, but it is usually taken to be equal to the decorrelation time of the MEG signal, i.e. the first zero crossing of the autocorrelation time of the signal. If the dynamics of the system under consideration is chaotic, the evolution of the system in the phase space, once transients die out, settles on a submanifold which is a fractal set, the strange attractor.

The concern of the strange attractors is of a great importance is chaotic dynamics, since its existence or absence is related to the behavior of the system as chaotic or deterministic.

If a strange attractor exists, it can be described by a geometrical parameter the correlation of fractal dimension D.

This parameter is related to the number of variables required to define the attractor within the phase space.

According to the method proposed by Grassberger and Procaccia (22), D can be estimated from an experimental time series by means of the correlation integrals C(r, m) defined as:

$$C(r,\ m)=\lim_{n\to\infty}(n(n-1)/2)^{-1}\sum_{i=1}^{n-1}\sum_{\substack{j=1+i\\ i\neq j}}^{n}\Theta(r-|V_i-V_j|)$$

(2)

where Θ(u) is the Heaviside function defined as (Θ(u)=1 for u>0 and Θ(u)=0 for u≤ 0), m is the embedding dimension and n is the number of vectors constructed from a time series with N samples, given by the formula $n=N-(m-1)\tau$ (here τ is a delay parameter which is equal to the first zero crossing of the autocorrelation time of the MEG signal). The correlation integral C(r, m) measures the spatial correlation of the points on the attractor and it is calculated for different values of r in the range from 0 to r_{max} . The r_{max} is equal to $(m)^{1/2}$ $(x_{max}-x_{min})$, (assuming that x_{max} and x_{min} are the maximum and the minimum recorded values in the time series). For a chaotic system the correlation integrals should scale as $C(r, m) \sim r^{D(m)}$. Thus, the correlation dimension D of the attracting submanifold in the reconstruction phase space is given by :

$$D=\lim_{\substack{r\to 0\\ m\to\infty}}\partial(\ln C(r,m))/\partial(\ln(r))$$

(3)

In the case of a chaotic signal exhibiting a strange attractor, there is a saturation value, (plateau) in the graph of the slopes ∂ (lnC(r, m)) / ∂ (ln(r)) vs ln(r). This value remains constant, although the signal is embedded in successively higher-dimensioned phase spaces and gives an estimation of the correlation dimension of the attractor.

Recording the MEG activity over the scalp in the case where the measurements are independent for each position (one-channel SQUID) requires that the MEG activity remains invariant in time. In order to ensure that the MEG activity was not influenced by long-term variations, we repeated the recordings at various positions at different times and found that there was very little difference in the measurements as such as 60 minutes apart during the experiments because there was a constancy in the D values. Thus the stability of MEG measurements in patients with CNS disorders justified in our view the use of a one-channel SQUID.

Using the above described method the correlation dimension D of the MEG time series for AD patients was estimated for the magnetic activity recorded from the AD patients and normal individuals using SQUID technology.

The purpose of this estimation was to investigate whether there is any biological differentiation in the dynamics in these two types of magnetic activities.

3. Results

In 9 AD patients examined the mean value of D was 10.02 with standard deviation 0.59 (D-range 9 to 11 as it is indicated in Table I). In addition to our studies we have included five normal individuals where the dimension D of the strange attractor shifted to higher values (13, 14).

In Fig. 2 and 3 present examples of the MEG time series obtained from the brain of AD patients and the

slopes of the correlation integrals (21, 22) which revealed the correlation dimension D (D=9.7) of the strange attractor.

Table I

The correlation dimension of 9 patients with Alzheimer Disease.

Subjects	Age	Correlation dimension
Women	69	9.7
	67	9
	72	9.9
	62	10.8
	55	11
	56	10
Men	66	10.1
	68	9.7
	72	10

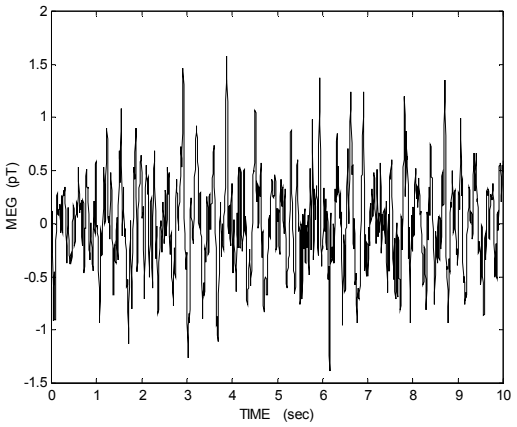

Figure 2

MEG time series for 10 sec duration obtained from a brain point of the AD patient.

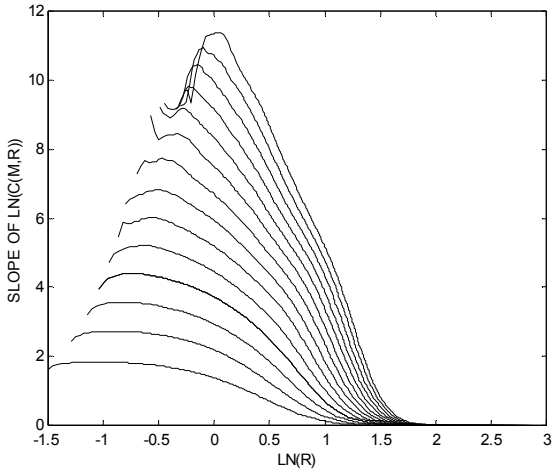

Figure 3

Plots of the slopes of the correlation integrals as a function of ln(r) for the MEG time series of Fig. 2.

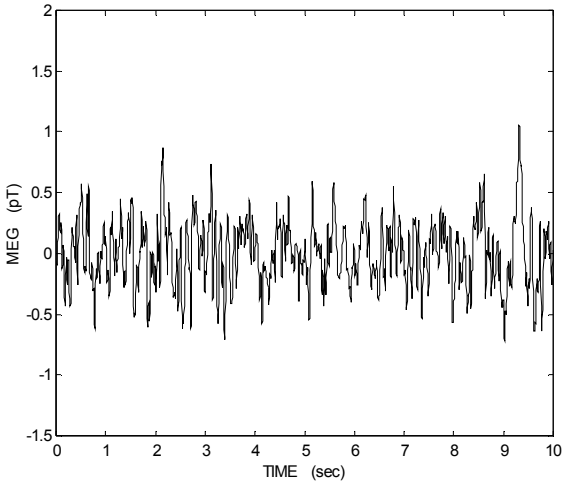

Figure 4

MEG time series for 10 sec duration obtained from a brain point of a normal subject for purpose of comparison.

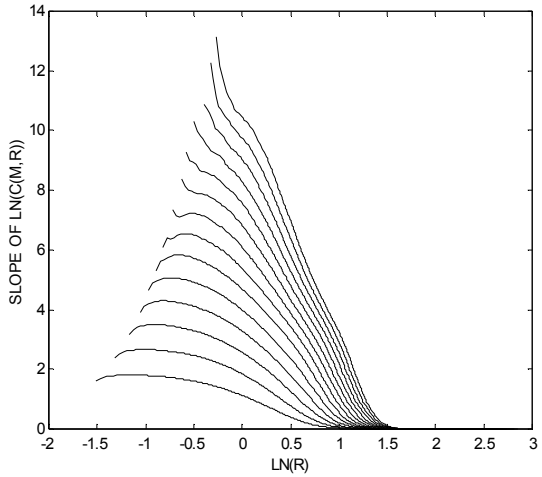

Figure 5

Plots of the slopes of the correlation integrals as a function of ln(r) for the MEG time series of Fig. 4.

Fig. 4 and 5 give MEG time series and the correlation dimension for normal subjects and we can see that we are dealing with a chaotic system, which is characterized with infinite value of D, in opposite to the findings for non chaotic system as is in the case of AD patients.

4.Discussion

This study is the first MEG recordings analysis from AD patients using non linear analysis and chaos. In simple statistics in which we have applied Fourier data analysis we found differences in spectral power including amplitude and frequency for a group of AD patients relative to age-matched controls (20). The increases in low frequency magnetic power values and decreases in high frequency power values is in agreement with the results of previous EEG studies in AD patients (25-35).

Our nonlinear techniques referred to the reconstruction of the phase space from the MEG time-series of the system, the detection of the existence of strange attractor and the estimation of its correlation dimension. This task is performed by analyzing the MEG time-series, which were recorded from different points of the right and left temporal lobes, or from the frontal and
occipital lobes of the brain of AD patients.

Dimensionality calculations (i.e. calculation of the correlation dimension of the strange attractor in the reconstructed phase space) can be utilized in the case of experimental biomedical signals, as is the case of MEG for the quantification of the complexity of the neuronal system under consideration. This is done using the embedding theorem (24) in order to reconstruct from a time-series of one dynamical component of the system, a topological equivalent phase space. Afterwards, the correlation integrals C(r,m) were calculated, for successively increasing values of the embedding dimension m, following the method of Grassberger and Procaccia (21, 22).

According to the dynamics of the system the observe MEG is a time series of $B_i=B(t_i)$ (i=1,2...N). Therefore, the vector construction V_i which is given by equation 1 is representative of the pathology of the AD patients. On the other hand this pathology is the outcome of the degeneracy of all the neurons which are involved in the above pathology.

Another point which is very important to discuss here is the estimation of the largest Lyapunov exponents as a function of the evolution time since we are dealing with time series MEG records. Lyapunov exponents provide a quantitative measure of chaos by describing the mean rate of divergence of initially neighboring trajectories as we defined above in the data analysis.

In most applications are needs only to measure the largest Lyapunov exponent, by examining the evolution of an infinitesimally small

displacement vector ξ_0 at a given point on the attractor.

For a chaotic system, the evolved vector ξ_t grows (on average) exponentially as $\xi_t = \xi_0 \cdot e^{\lambda \cdot t}$, $\lambda > 0$. The largest Lyapunov exponent λ is positive for a chaotic system. An adaptation of this procedure for determiny the Lyapunov exponent from experimental data set was originally proposed by Wolf et al. (36).

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The SQUID as Diagnostic Tool to Evaluate the Effect of Transcranial Magnetic Stimulation in Patients with CNS Disorders

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Abstract: Magnetoencephalograph (MEG) recordings of patients with CNS disorders were obtained using a whole-head 122-channel magnetometer SQUID and analyzed using Fourier statistical analysis. External transcranial magnetic stimulation in the order of pico Tesla (pTMS) was applied on the above patients with proper characteristics (magnetic field amplitude :1-7.5pT, frequency :the α-rhythm of the patient: 8-13 Hz) which were obtained with MEG recordings prior to pTMS. The MEG recordings after the application of pTMS shown a rapid attenuation of the high abnormal activity followed by an increase of the low frequency components toward the patients α-rhythm.

Key- Words: - SQUID, MEG, Parkinson, Epilepsy, Multiple Sclerosis

1 Introduction

The magnetic activity of the brain isproduced by cellular micro-currents, which emerge from ionic movements, due to the dynamical variations of the membrane potentials [1]. Even though transmembrane, intracellular and extracellular neuronal currents each produce surrounding magnetic flux, the neuromagnetic field recordable outside of the head is a selective reflection of intracellular currents flowing in the apical dendrites of pyramidal cells parallel to the skull surface. The magnetic field generated by a single neuron is almost negligible; however, when several thousands of nearby cells are synchronously active, the summated extracranial magnetic field typically achieves a magnitude of only a few hundred femto-Tesla ($1fT=10^{-15}$) where the strongest neuromagnetic signals like those associated with epileptic spikes are only a few thousands femto-Tesla in magnitude [1-4]. These magnetic signals can be measured with the use of sensors that take advantage of how the strength of a magnetic field changes as a function of the distance from its source. Such magnetic fields emitted from the brain are very weak (of the order of pT; $1pT=10^{-12}T$), so very sophisticated devices must be utilized in order to detect and record these fields .These devices are the ones which are based on the Josephson effect of superconductivity [5]. Such sophisticated device is the magnetometer SQUID the name of which comes from the initials of the following words (Superconductive Quantum Interference Device). The SQUID has the ability to detect magnetic fields of the order of 10^{-15} T which is much smaller than the magnetic field of the earth which is $5X10^{-5}$ T or 50μT. The signal measured by each channel of the magnetometer SQUID is a time varying waveform voltage that reflects local changes in the magnetic flux as a function of time. This signal is called magnetoencephalogram (MEG) if we measured the brain emitted magnetic fields and it is very similar to the electroencephalogram (EEG) if we measured the brain emitted electric fields.

The MEG is presently regarded as the most efficient method for recording the brain activity in real time for many reasons. Compared with the EEG, the MEG has unique sensitivity to the CNS disorders and normal functions of the brain. In addition, the MEG offers functional mapping information and measurement of brain activity in real time, unlike CT and MRI and fMRI which only provide structural, anatomical and metabolic information. With the MEG the brain is seen in 'action' rather than viewed as a still image. Last, and most important is that the MEG has far more superior ability to resolve millisecond temporal activity associated with the processing of information which is the main task of the brain.

Thus, both normal spontaneous rhythms and pathological activities are readily identified in MEG waveforms as we do with the EEG waveforms. Whereas, MEG signals reflect current

flow in the apical dentrites of pyramidal cells oriented tangential to the skull surface, EEG signals reflect both tangential and radial activities [6].
The goal of this review was to report the above mentioned potential
superiority of the MEG signals obtained in the diagnostic evaluation of CNS patients before and after the application of low intensity external transcranial magnetic stimulation using Fourier statistical analysis in frequency domain.
The transcranial magnetic stimulation (TMS) as currently used, was introduced by Barker et al. [7]. The TMS provided for the first time as a non-invasive, safe and painless method of activating the human motor cortex and assessing the integrity of central motor pathways. Since its introduction, the use of TMS in clinical neurophysiology, neurology, neuroscience and psychiatry has spread widely, mostly in research applications, but increasingly with clinical aims in mind [8,9]. On the other hand Anninos and his associates [3,4,10] applied also with a special electronic device [11] weak external TMS (in the order of pico Tesla) with proper field characteristics (intensity : 1-7.5 pT, frequency : 8-13 Hz) in the frontal, occipital and temporal lobes of the patients with CNS disorders. This electronic device consists of a low voltage generator, which can produce low frequencies, from 2-13 Hz, to a group of 32 coils of 1cm in diameter [11]. The 32 coils are enclosed between two parallel plastic plane surfaces in such a way that the axis of the coils is situated perpendicular to these surfaces.
The TMS can be applied as single pulses of stimulation, pairs of stimuli separated by variable intervals to the same of different brain areas, or as trains of repetitive stimuli at various frequencies.
Single stimuli can depolarize neurons and evoke measurable effects. Repetitive TMS can modify excitability of the cerebral cortex at the stimulated site and also at remote areas along functional anatomical connections [12]. With this new medical tool we ought to ask ourselves what it can be offered that established methods do not for diagnostic, prognostic and therapeutic parts of clinical neurology. A new neurological tool might have several benefits: establishment of a differential diagnosis earlier or with greater certainty for a given clinical presentation than existing methods; better prediction of the likely course of the disease; further support for sustained and intensive interventions; help in identification of the most suitable treatment strategy; or improvement of clinical outcome as a therapy itself.

The main clinical application of TMS concerns testing of the functional integrity of the corticospinal tract in patients with disorders affecting the CNS. Use of standard TMS in these neurological disorders provides information on detection of subclinical upper motoneuron involvement, localization of anatomical site of lesions, longitudinal monitoring of motor abnormalities during course of diseases, and valuable aid to differential diagnosis. Repetitive stimulation of the brain opens a new field of investigations of cognitive function and mood and therapeutic possibilities. There are interesting results in the short-term treatment of refractory depression by daily sessions of repetitive TMS. By changing the frequency of stimulation, it may be possible to modulate cortical excitability for therapeutic benefit. Thus, the ability of TMS to measure and modify cortical activity offers possibilities to apply this methodology to clinical neurology, neurorehabilitation and psychiatry [13].

2.TMS in clinical Neurology

The TMS has been tested to study different forms of epilepsies from generalized to focal epilepsies. The most common abnormality in all types of epilepsies that we have studied was an increased excitability with a reduction of intracortical inhibitory mechanisms. In order to test the effect of the application of TMS to all these types of epilepsies MEG measurements were performed using the whole-head 122-channel SQUID gradiometer device operated at low liquid helium temperatures (4K0). Recordings were taken in an electromagnetically shielded room in order to avoid extraneous electromagnetic noise. The MEG recordings were obtained with sampling frequency of 256Hz and filtered with cut-off frequencies between 0.3 to 40 Hz. The time taken for each recording was 2min in order to ensure alertness for each subject.
A software program was developed in our lab in order to detect the primary dominant frequency of the power spectra of the MEG obtained from each channel after the application of Fast Fourier Transform for each epileptic patient. Then, it was constructed a two dimension map for the spatial distribution of the above mentioned primary dominant frequencies over the scalp. Different colors in the map represent different dominant frequencies (red=2Hz, pink=3Hz, yellow=4Hz, green=5Hz, blue≥6Hz). Figures 1 and 2 respectively demonstrate the maps of the spatial distribution of the 1st dominant frequency over the scalp for a particular epileptic patient randomly

selected from a large pool of epileptic patients and a normal volunteer before the application of external pTMS. As it is observed prominent low frequencies can be seen in the map for the epileptic patient, whereas in the control volunteer map show that the frequency range was ≥6 Hz in the majority of channels indicating the appearance of a-rhythm which is the control rhythm for normal subjects. Thus, the spatial distribution of the power frequency amplitude in the maps of all examined epileptic patients tend to be located over a wide area in the low frequency domain, whereas in normal subjects the spatial distribution of the power frequency amplitude is clustered in the map showing domains with higher frequency.

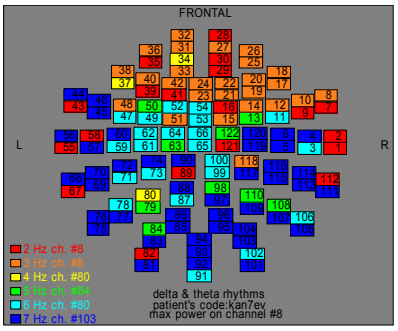

Figure 1. This figure gives the spatial distribution of the first dominant power frequency amplitude for one epileptic patient in which it is seen prominent low frequencies in most of the brain areas.

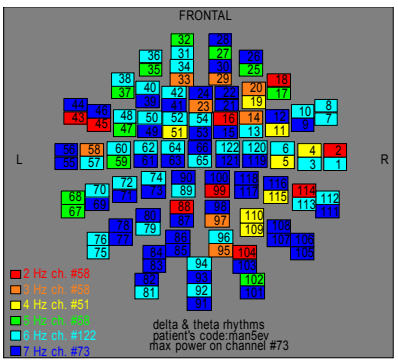

Figure 2. This figure shows the spatial distribution of the power spectra for the first dominant frequency amplitude from a normal subject. In this map it is observed in all brain regions prominent frequencies ≥6Hz.

There are interesting results in the short-term treatment by daily sessions by applying TMS(magnetic intensity:1-7.5pT; frequency: the a-rhythm of the patient:8-13Hz) in all epileptic patients including the randomly selected one stated above. This was done by placing the coils of the device [11] on the patient's scalp for a total of 6 minutes (2 minutes over each of the following areas: left and right temporal regions, frontal and occipital regions, and over the vertex). The time between the first MEG and the MEG obtained after the application of the TMS was about one hour. By applying the same shoftware program, as it was stated above, we can detect the primary dominant frequency of the power spectra of the MEG records obtained from each channel after the application of TMS for the selected epileptic patient. Then, it was constructed a similar map for the spatial distribution of the primary dominant frequencies over the scalp.

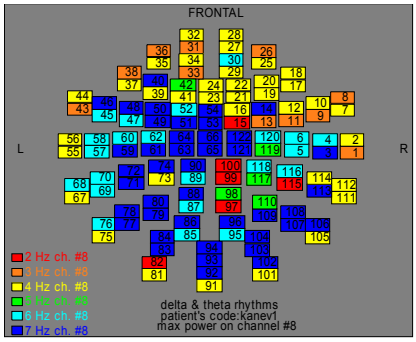

Figure 3. This figure shows the spatial distribution of the power spectra for the first dominant frequency amplitude from the MEG records after TMS for the epileptic patient of Fig.1.

Similar studies we have performed also with Parkinson's disease patients before and after the application of external transcranial magnetic stimulation. All the Parkinson patients had diagnosed independently to suffer from idiopathic Parkinson disease (PD) and none of the patients had a history of other neurological disease other than PD. Biomagnetic MEG measurements were performed, as before using the whole-head biomagnetomer 122 channel SQUID in a magnetically shielding room of low magnetic noise. During the MEG recordings the subjects, as before, were sitting in a chair with their heads covered by a helmet shaped dewar. Four indicators coils attached to the patient head determined the exact position of the head with respect to the MEG sensors.
The exact positions of the coils were determined using a three dimensional digitizer. In Figure 4 it is shown the spatial distribution of the power spectra for the first dominant frequency amplitude obtained from the MEG records from a particular PD patient

selected randomly from the pool of all examined Parkinson patients prior to the application of external magnetic stimulation.

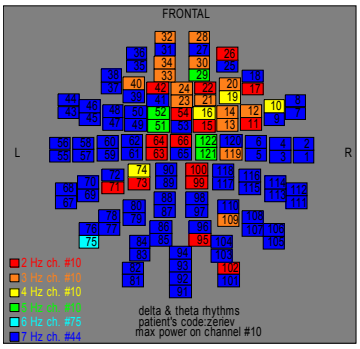

Figure 4. The spatial distribution of the power spectra for the first dominant amplitude frequency obtained from the MEG records of a Parkinson patient before TMS.

As it can be seen from Fig.4 the spatial distribution of the power spectra for the first dominant frequency is characterized by low frequencies. On the other hand the application of external magnetic stimulation on the Parkinson patient of Fig.4, as it is seen in Figure 5 shows that the power spectra distribution of the first frequency amplitude is cluster in domains showing higher frequency as it should be for normal subjects.

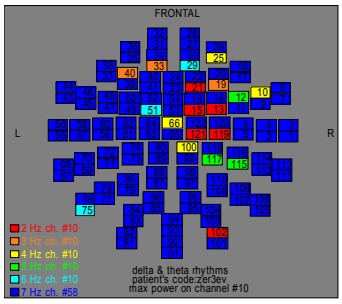

Figure 5. This figure is showing the distribution of the power spectra for the first dominant frequency amplitude of the MEG records obtained from the Parkinson patient of fig.4 after the application of TMS.

To confirm that the responses to TMS were reproducible, as it is shown in Fig.3 and fig.5, the patients were instructed to apply TMS with the same characteristics, with those used in our laboratory, nightly at home. Since this resulted in the same reaction to the one obtained in our laboratory and since this effect was sustained for a period more than a year, we preliminarily concluded that the application of the TMS is a non-invasive, safe and efficacious modality in managing patients with CNS disorders.

3. Results

The results reported in this section are representative for the group of epileptic and Parkinson patients that were diagnosed for the last five years using the whole-head 122 channel SQUID. The first case presented here refers to 30-years old patient suffering from idiopathic epilepsy since the age of 11. When he was first visited our Laboratory (in September 2001), he was manifesting five to 10 seizures per day with loss of consciousness and without falling down. The use of MEG recordings with the 122 channel SQUID diagnosed as having generalized epilepsy as it is seen in the map of Figure 1. This figure is showing the spatial distribution of the power spectra for the first dominant frequency amplitude of the MEG recordings obtained from the scalp of the patient prior to the external magnetic stimulation.

After the application of external magnetic stimulation to the above epileptic patient, using the electronic device [11] with the specific characteristics in the field intensity and frequency, as were stated in the introduction, we can obtain again a new MEG record.

Figure 3 illustrates the effect of the spatial distribution for the power spectra of the first dominant frequency amplitude for this randomly selected epileptic patient from the pool of epileptics patients examined in our laboratory. As it is seen the new map is characterized by a cluster of higher frequencies similar to the map of normal subjects. In addition, we have seen that this procedure was associated with the attenuation in the frequency and severity of patients seizures.

The second case is for a Parkinson patient selected also randomly from the group of Parkinson patients diagnosed in our laboratory. All these patients have diagnosed to suffer from idiopathic tremor, rigidity, and dyskinesia on the basis of clinical observations and routine EEG recordings. The use of MEG recordings again with the whole-head 122 channel SQUID we obtained the map of Figure 4. This map is showing the spatial distribution of the power spectra for the first dominant frequency amplitude of the MEG recordings obtained from the Parkinson patient scalp prior to the external magnetic stimulation. This Parkinson patient was selected randomly from the whole group of

Parkinson patients diagnosed with the 122 channel SQUID in our laboratory.
In this map we noticed that there are certain areas where the first dominant frequency amplitude, obtained from the power spectra of the MEG recordings from the scalp of the above mentioned Parkinson patient, are showing domains of low frequency . After the application of external magnetic stimulation to the above selected Parkinson patient we can record again a new MEG as before.
Figure 5 illustrates again the effect of the spatial distribution for the power spectra of the first dominant frequency amplitude which is characterized by similar cluster of higher frequencies similar to the map seen in normal subjects. Furthemore, it was noticed that with this procedure the Parkinson patients resulted in rapid attenuation of Parkinson symptoms.

4. Discussion

The brain is a complex dynamical system, so multichannel measurements are necessary to gain a detailed understanding of its behavior. Such multichannel measurements include optical brain images, multielectrode recordings, functional magnetic resonance imaging, MEG, etc[14-16].
In the MEG recordings, weak magnetic fields of the order of tens of fT/√Hz generated by electric currents in the brain are measured using the SQUID's detectors placed on the skull of the patients. The MEG is a noninvasive imaging technique, applicable to the human brain with temporal resolution approximately ~1ms [17]. Several authors have demonstrated the importance of the MEG in the investigation of normal and pathological brain conditions during the last decade [18-22]. The major advantage of MEG over EEG is that MEG has higher localization accuracy. This is due to the fact that different structures of the head (brain, cerebrospinal fluid, skull and scalp) influence the magnetic fields less than they influence the volume current flow that causes the EEG. Additionally, the MEG is reference free, so that the localization of the sources with a given precision is easier for the MEG than it is for EEG [23].
Frequency analysis is being increasingly applied in the investigation of CNS disorders before and after the application of low external magnetic fields with several advantages over the time domain technique [24]. Low frequency activities have been observed in our maps and occurred as thalamocortical synchronization transiently during wakefulness, under specific conditions of mental and emotional activity. Comparing all the maps which were obtained from the spatial distribution of the power MEG spectra for the first dominant frequency amplitude from all subjects, it can be seen that there is an increase number in the low first dominant frequency power amplitude for all subjects before the application of TMS, whereas the opposite is true for all subjects after the application of TMS.
Therefore, due to this beneficiary effect, the application of such external magnetic fields has been used recently by more and more scientists using transcranial and intracranial methodologies and have become convinced that it is proven to be a valuable tool for managing CNS disorders [25-27].

5. Conclusion

Although the beneficial effects of the application of TMS on the clinical picture in all CNS patients are well observed, the mechanisms underlying the efficacy of TMS remains an open question. One possible explanation of our findings is provided indirect support of our hypothesis that the beneficiary properties of the TMS are mediated via the pineal gland which is a magnetosensitive organ of our brain [3]. Taking this into account, the activity of this gland may be one of the crucial factors which determine and control the neural activity of all these patients suffering from CNS disorders. However, the question is difficult to be answered given the complexity of cellular, systemic and neuroendocrine effects of the TMS on biological systems and their potential impact on neurotransmitter functions. Despite all these facts, this method of magnetic stimulation may be considered as a very important noninvasive modality in the management of idiopathic CNS disorders.

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Magnetoencephalographic Analysis and Magnetic Stimulation in Patients with Alzheimer Disease

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Abstract. Background. The magnetoencephalogram (MEG) is the magnetic activity emitted by the brain, which can be measured using a Superconductive Quantum Interference Device (SQUID). This method is non-invasive and MEG recordings were obtained from patients suffering from Alzheimer disease (AD).
Method. The emitted magnetic activity was recorded from a total of 64 points of the skull (32 points from each temporal lobe). This MEG signals analyzed using Fourier statistical analysis and the spatial distribution of the magnetic power spectral amplitudes over the scalp was calculated. Following this calculation, ISO-SA maps were constructed from the average power spectral amplitudes in the 2-7 Hz frequency range. Using this method it is easy to detect the existence of abnormal points, due to the fact that ISO-SA maps represent pictorially the projected neural brain activity on the scalp. Furthermore, external magnetic stimulation (EMS) with intensity 1-7.5pT, and frequency the α-rhythm of the patient (8-13 Hz) was applied in the left-right temporal, frontal-occipital and vertex regions and the emitted brain magnetic activity was recorded again.
Results. Some of the recorded points were observed to exhibit abnormal rhythmic activity, characterized by high amplitudes and low frequencies. The application of the EMS resulted in rapid attenuation of the abnormal brain activity of AD patients.
Conclusions. The findings that application of external magnetic fields of low intensities and frequencies produce attenuation in abnormal activity in AD patients indicates that this novel method may open new ways in AD treatment.

Key Words: magnetic stimulation, Alzheimer patients, MEG

1. Introduction

Alzheimer disease (AD) is a multifactorial [1] and a progressive degenerative disease of the central nervous system (CNS) and also the main cause of dementia in elderly people over 60 years of age.[2] Several microscopic changes take place in the brains of Alzheimer patients. The pathological hallmarks of Alzheimer's disease brains is amyloid plaques, neurofibrillary tangles (bundles of abnormal filaments within neurons), deficits in several neurotransmitter systems, neuronal cell loss and changes in neuronal morphology.

Therefore, it is attempting to use a novel technology in order to achieve a better understanding of AD. The neuronal populations in the brain emit spontaneous magnetic activity caused by ionic movements across the plasma membrane.[3] [4] This neuronal activity, although exceedingly weak (it is about 10^{-8} of the earth's magnetic field which is equivalent to 50 μT), can be measured by means of a superconducting quantum interference device (SQUID). The SQUID is a diagnostic tool capable of measuring the exceedingly weak magnetic fields emitted by the neurons in the brain. The recordings of these fields, which are called Magnetoencephalogram's (MEG's), are very interesting in the investigation of normal and pathological conditions of the brain, like epilepsy, Parkinson disease and other dysfunctions of CNS.[5] [6] The method is non-invasive because the SQUID is a receiver and not a transmitter. Here we report the potential value of the biomagnetometer SQUID and the use of Fourier statistical analysis in assessing AD.

Furthermore, we applied external magnetic stimulation (EMS) on the points that were found to exhibit abnormal rhythmic activity characterized by high amplitudes and low frequencies. The applied EMS consisted of magnetic fields with intensity in the pT range and α-rhythm frequencies in the range 8-13 Hz. MEG recordings shown a rapid attenuation of the high abnormal MEG activity followed by an increase of the low frequencies components toward the α-rhythm after the application of the EMS as we can see from our recent publications. [5] [7-9] Further signal analysis findings indicated that the application of the EMS strongly influenced the underlying brain dynamics

with beneficial effects on the clinical status of the Alzheimer patients.

2. Materials and Methods

Subjects

AD patients were referred to the Laboratory of Medical Physics, by practicing neurologists. All patients had been diagnosed independently to suffer from AD. Patients had normal routine serum biochemical studies, as well as, normal CT or MRI scans. In all cases informed consent for the methodology and the aim of the study was obtained from all patients prior to the procedure. The Hospital's Ethics Committee has approved this study. MEG recordings were obtained from 9 patients with AD. The age of the AD patients ranged from 55 to 72 (mean=65.2, SD=6.3).

Data acquisition, analysis and brain mapping

We used a single channel SQUID second order gradiometer (DC SQUID model 601 of the Biomagnetic Technologies), which was constructed in USA. MEG recordings were taken in an electrically shielded room with the patient lying supine on a wooden bed, free of any metallic object, so as to decrease the environmental noise and get better S/N ratio. The magnetic activity was recorded from the right and left temporal lobes and sometimes from the frontal and occipital lobes. For each lobe 32 equally spaced matrix points were recorded, properly located with respect to the T3 point of the International 10-20 Electrode Placement System [10] in the case of the left temporal lobe (Figure 1), the T4 in the case of the right temporal lobe, P3 and P4 in the case of the occipital lobes and F3 and F4 in the case of the frontal lobes. MEG recordings were performed after positioning the SQUID sensor 3 mm above the scalp of the patient.

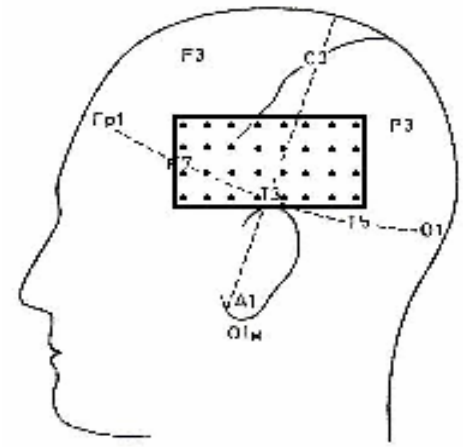


Fig. 1.—This figure is showing the 10-20 International Point System the points of which are served as origin in our rectangular reference system

For each one of the 32 matrix points, at least 32

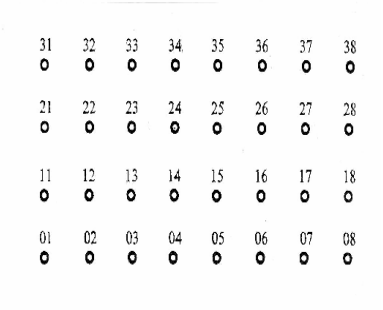


Fig. 2.—The 32 matrix points in our rectangular reference system

consecutive MEG epochs were recorded, of 1 sec duration each. The position for the MEG recording points takes less than a minute and we start either with the 38 matrix point or with the 31 matrix point (Figure 2) depending of whether we measure the left or the right hemisphere respectively. Then by using an automatic optic point position system, constructed in our Laboratory, we can record all the other points in the matrix. The total recording time required for one hemisphere of 32 matrix points is about 30 minutes. Therefore for the two temporal hemispheres the total time is about 1 hour.

For the two MEG measurements prior and after magnetic stimulation it takes about 2 hours. With respect to the status of the patient due to the 2 hour evaluation procedure we state that this procedure does not continue for the whole period of MEG measurements but it is performed in steps. Thus, not only we have to turn the patient from the left to right hemisphere but also due to the fact that all 32 points are not all located in the same level on the skull of the patient it is required to re-position the SQUID every 16 points for each hemisphere. The acquired MEG records were digitized using a 12 bit precision analog to digital converter with a sampling frequency of 256 Hz, and were stored in a PC peripheral memory for off-line statistical analysis.

The MEG data of the 32 points of each lobe were analyzed using Fourier statistical analysis and the spatial distribution of the magnetic power spectral amplitudes over the scalp was calculated. Following this calculation, ISO-SA

maps (which represent the lines of the locus of all points with equal power spectra amplitudes for a particular frequency or frequency range) were constructed from the average power spectral amplitudes in three different frequency bands: compound θ and δ rhythms (2-7 Hz), α rhythm (8-13 Hz) and β rhythm (14-25 Hz). Using this method it is easy to detect the existence of abnormal points, due to the fact that ISO-SA maps represent pictorially the projected localizations of the neural brain activity on the scalp.

External magnetic stimulation (EMS) was applied in the temporal lobes on the 32 - point matrix (4.5x10.5 cm rectangle) of the patients using an electronic device [11], and the emitted magnetic activity of these points of the brain was recorded again. The applied EMS carried similar field characteristics (magnetic field in the range of 1-7,5pT and frequency the α-rhythm of the patient (8-13 Hz)) with the ones emitted from the brain prior of the application of EMS. The time between the 1st MEG and post-stimulation MEG is about an hour. In all patients placebo tests were also performed using the electronic device [11] without being energized and the emitted magnetic activity of all points was recorded again without any substantial changes from the MEG activity recorded prior to magnetic stimulation. None of the patients experienced side effects during or after the procedure.

3.Results

In Figure 3a is shown the wave-form (raw data) of the left temporal area of the AD patient before EMS. This particular MEG record it is characterized by high amplitudes and rhythmicity (the maximum power amplitude is 2,2pT).

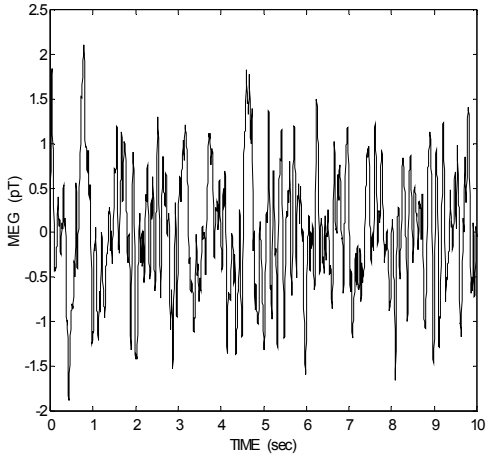
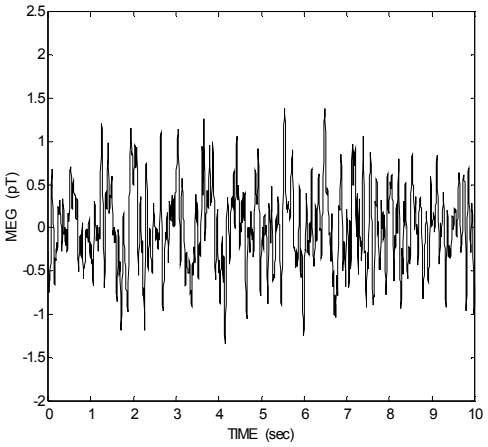


Fig. 3a.—MEG time series for 10 sec duration time obtained from a brain point of the left temporal area of the AD patient before magnetic stimulation. The maximum power amplitude is 2,2pT

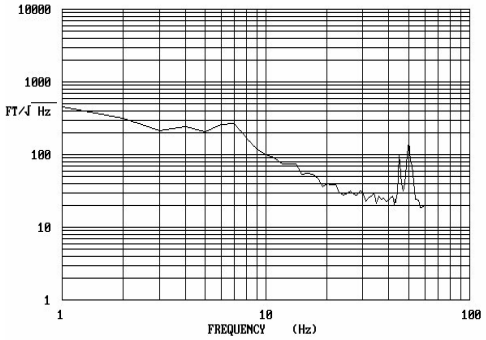
The Fourier Power Spectrum estimated by applying a FFT algorithm on the MEG data of Figure 3a is illustrated in Figure 4a, which is exhibiting its maximum value at 2 Hz frequency. In Figure 3b-4b are presented the corresponding plots to Figures 3a-4a, which were obtained analyzing the MEG data obtained from the same patient and the same area, after EMS.

Fig. 3b.—MEG time series for 10 sec duration time obtained from the same point of the AD patient after magnetic stimulation. The maximum power amplitude is reduced to 1,4pT.



As it shown in Figure 3b the MEG obtained in this way does not exhibit high amplitudes and rhythmicity (the maximum power amplitude is 1,4pT).

Fig. 4a.— The power spectrum of a point of left temporal area of the AD patient before magnetic stimulation. The maximum power amplitude in 2 Hz is 310 fT/√Hz.



The resulting Fourier Power Spectrum estimated by applying a FFT algorithm on the MEG data of Figure 3b is illustrated in Figure 4b. As it is shown in Figure 4b (the frequency is 6 Hz) which is considerably different compared to the one shown in Figure 4a which is 2Hz (its amplitude is higher than the 7 Hz), which corresponds to the MEG recorder prior to the application of the EMS. In Figure 5a is shown the ISO-SA maps of our AD patient before EMS. The maximal total average emitted power in the 2-7 Hz band frequency is considerably high. In Figure 5b in the ISO-SA maps after EMS, we have the maximal total average emitted power in the same frequency band. As we can see the value of power is lower than the power before EMS.

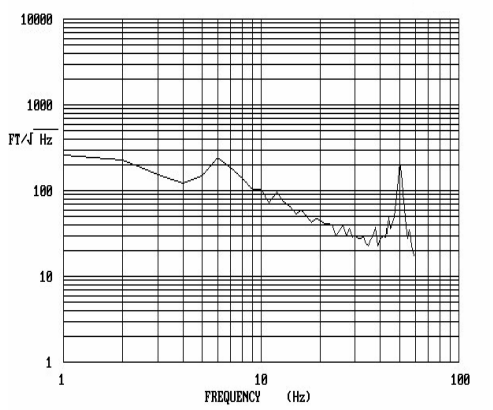


Fig 4b.- The power spectrum of a point of left Temporal area of the same patient after magnetic stimulation. The maximum power amplitude in 6 Hz is about 250fT/√Hz

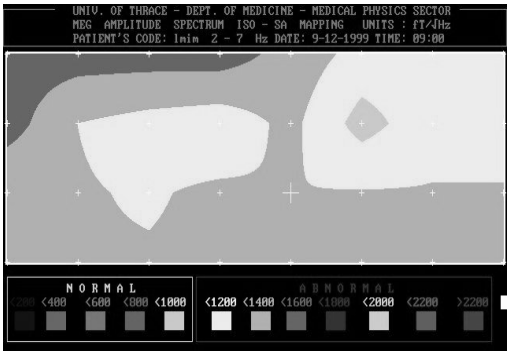


Fig. 5a.—The ISO-SA map of the left temporal area of the patient before magnetic stimulation. The emitted power in the 2-7 Hz band frequency is considerably high.

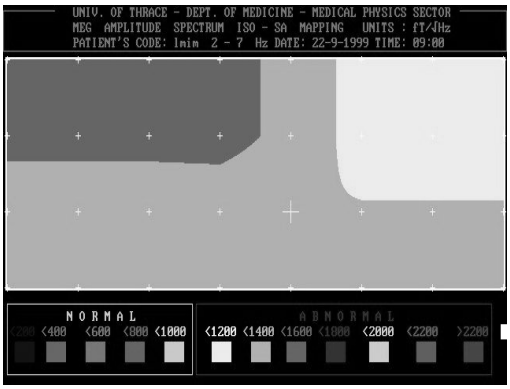


Fig. 5b.—The ISO-SA map of the left temporal area of the same patient after magnetic stimulation. The emitted power in the 2-7 Hz band frequency is reduced.

4. Discussion

The brain of AD patients emits MEG activity characterized by high amplitudes and rhythmicity. With the use of ISO-SA maps we were able to observe areas of high spectral density in the 2-7 Hz band frequency. In addition, the ISO-SA maps were helpful in providing clear identification of the coordinates of the points on the scalp where their MEG power spectrum has its maximal power as well as its maximal magnetic field intensity in the frequency range 2-7 Hz. Mapping the spectral power distribution over a surface, in the case where the measurements are independently recorded for each position (as in our case where a single channel SQUID is used), requires that the recorded MEG activity remains invariant in time. In order to ensure that in the course of our recordings the MEG activity was not influenced by long-term variations, we repeated the recordings at various positions at different times and were found that indeed there was very little difference in the power spectrum between records as much as 60 minutes apart during the experiments.[7 12] Thus, the stability of MEG measurements in patients with CNS disorders justified, in our view, the use of a one channel SQUID.

In addition to ISO-SA mapping technique, in AD patients we also applied artificial magnetic fields back to the brain in an attempt to attenuate the abnormal activity.[5 7-9] The information obtained from each point regarding the emitted magnetic field intensity, frequency and coordinates was

subsequently stored in a special integrated circuit which was used to energize an electronic device. [7 9 11] The latter was used to emit back magnetic fields of proper intensities and frequencies to the previously defined points. To confirm that the above responses to magnetic treatment were reproducible, the patients were instructed to apply magnetic fields using the electronic device nightly at home. Since this resulted in the same reaction to the one obtained in our laboratory and since this effect was sustained for a period more than a year, we preliminarily concluded that the application of weak external magnetic fields is a non-invasive, safe and efficacious modality in the management of abnormal activity in AD patients.

The possible mechanisms by which magnetic fields have attenuated the patient's symptoms are still controversial. However, it is known that magnetic fields have been shown to alter the activity of the pineal gland, which in turn, has been shown to regulate dopaminergic,[13] 5-HT,[14] GABA,[15] and endogenous opioid functions.[16] Moreover, in laboratory animals application of artificial magnetic fields has been shown to alter the activity of neurons in the habenular complex,[17] a structure which has been shown to function as a "gate" through which limbic dopaminergic systems interact with mid-brain/striatal dopaminergic neurons.[2 18] Furthermore, exposure of an organism or biological material to magnetic fields has been reported to induce mutagenic,[19] immunological,[20] metabolic,[21] endocrine,[22] morphological,[23] developmental,[24] behavioural,[25] [26] and anticonvulsant [7 27] effects. On a cellular level, the effects of magnetic fields maybe related to alterations in properties and stability of biological membranes as well as their transport characteristics including the intra- and extracellular distributions and flux of calcium ions.[27]

In conclusion, the findings that application of external magnetic fields of low intensities and frequencies produce attenuation in abnormal activity in AD patients indicates that this novel method may open new ways in AD treatment.

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The use of the Biomagnetometer SQUID to Evaluate the pTMS in Patients with CNS Disorders

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Abstract: Magnetoencephalographic (MEG) recordings in patients with CNS disorders were obtained in our lab for more than 20 years using a single and more recently a 122- channel magnetometer SQUID and analyzed with linear signal Fourier statistical analysis. External transcranial magnetic stimulation in the order of pico Tesla (pTMS) was applied on the above patients with proper field characteristics (Intensity: 1-7.5pT, frequency: the α – rhythm of the patient: 8-13Hz) with the ones obtained prior to pTMS. The MEG recordings after the application of pTMS were shown a rapid attenuation of the abnormal brain activity followed by an increase of the low frequency components toward the patients' α – rhythm. In addition were seen improvement and normalization of their EEGs and MEGs.

Keywords: MEG, magnetic stimulation, CNS disorders

1.Introduction

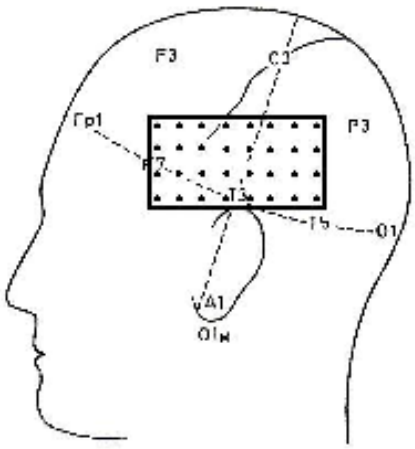
Time varying electric currents, in wires or brain cells, all produce time-varying magnetic fields (**1**). Even though transmembrane, intracellular and extracellular neuronal currents each produce surrounding magnetic flux, the neuromagnetic fields recordable outside of the head are a selective reflection of intracellular currents flowing in the apical dendrites of pyramidal cells oriented parallel to the skull surface. In contrast, cortical neurons which are oriented perpendicular to the scalp surface do not contribute to the extracranial magnetic fields perpendicular to the scalp (**2**).

The magnetic field generated by a single neuron is almost negligible; however, when several thousands of nearby cells are synchronously active, the summated extracranial magnetic field typically achieves a magnitude of only a few hundred femto-Tesla ($1fT=10^{-15}$), where the strongest neuromagnetic signals – those associated with epileptic spikes or other abnormal CNS disorders are only a few thousands femto - Tesla in magnitude (**2,3,4**).
This is still more than one billion times smaller than the earth's steady magnetic field ($3X10^{-5}T$) and the noise fields generated by even distant moving metal objects (e.g cars, elevators and so on) and power lines.
The detection and isolation of such neuromagnetic signals was a challenging problem. Thus, these magnetic fields, which as we have said before are very weak, in order to be detected we need very sensitive and sophisticated devices. Such sophisticated devices are the ones which are based on the Josephson effect of superconductivity (**5**) and which are called SQUID's from the initials of the four words (Superconductive Quantum Interference Device). The SQUID is in operation mode when it is in superconducting state and it becomes superconducting when their sensors are immersed in liquid helium contained in a large thermos or dewars. The liquid helium cools the SQUID's sensor to 4^0 Kelvin (-269^0 C) or 4^0 C above absolute zero temperature. The SQUID has the ability to detect magnetic fields of the order of $10^{-15}T$ (=1fT). The signal measured by the SQUID is a time varying voltage waveform that reflects local changes in the magnetic flux as a function of time. This signal is called magnetoencephalogram(MEG) if it is measured the brain emitted magnetic fields and it is very similar to the electroencephalogram(EEG) which is measured the brain emitted electric fields((6,7,8,9).
the apical dendrites of pyramidal cells, as it was mentioned before, which are oriented tangential to the skull surface, the EEG signals reflect both tangential and radial activities (**10**).

Thus, both normal spontaneous rhythms and pathological activities are readily identified in MEG waveforms as it is with the EEG waveforms (**10**). Whereas MEG signals reflect current flow in

Since the principal generator of the MEG lies in the layer of pyramidal cells and the MEG is produced exclusively by a flow of electric current tangential to the skull surface, it appears that the signal will originate maximally from the cerebral sulci (where the pyramidal cells are more favorably oriented) and only minimally from the surface of the gyri where their orientation is less favorable (**3**). MEG measures brain currents without physical contact with the skull, is also insensitive to the conductivities of the scalp, skull, and brain, which as it is known affects EEG measurements (**10**).

The information provided by the MEG is entirely different from that provided by Computed Tomography (CT) or Magnetic Resonance Imaging (MRI). Unlike the latter two, which provide structural/anatomical information, MEG provides functional mapping information. MEG is a powerful functional imaging technique complementary to the anatomical imaging capabilities provided by the MRI and CT. That is, whereas MRI and CT are capable of imaging anatomy, MEG is able to image neurological function. Another important characteristic point of the MEG is that it is measuring the activity of the brain in real time. The brain can be observed "in action" rather than just viewing a still MRI or CT image. The MEG data obtained with the SQUID techniques can be used to identify both normal and abnormal functions of brain structures, which are anatomically so crispy seen in the static, MRI and CT scans. As a result, the MEG technique is very useful technique for clinical use. Thus, the MEG measurements associated with the neural currents in the brain can be used to diagnose epilepsy, mental illness, Parkinson, as well as to study brain function (**2,3,4,11,12**).



Thus, the goal of this chapter is the potential use of the SQUID and the MEG measurements in diagnostic evaluation in patients with CNS disorders before and after the application of external transcranial magnetic stimulation in the order of pico Tesla (pTMS), which is a method invented for first time by the authors in 1989 (**13**).

The potential use of transcranial magnetic stimulation to modulate learning, memory, higher cognitive human functions and seizures, was applied also afterward by other investigators (**14,15,16**).

2. Methods and Results

The external magnetic fields was applied first to patients with seizure disorders in an attempt to attenuate seizure activity (**13**). That proposal seemed to be justified in view of the observations in animals, which showed that administration of low-frequency magnetic fields altered the electrical activity in the brain and, thus, influenced the frequency or severity of seizures (**17**). Furthermore, was reported an inhibitory effect of low-intensity 60 Hz magnetic field exposure on electrically kindled seizures in rats and humans (**17**).

Over the past fifteen years we have applied external magnetic stimulation and evaluated their MEG prior and after pTMS in more than 1000 patients for treatment of various forms of epilepsy (**3,4,18**). All patients had been resistant or refractory to conventional anticonvulsant therapy and exhibited frequent seizures over a long period of time. Thus, using a single channel SQUID the MEG activity was recorded from each of the 32 rectangular matrix points (4 rows X 8 columns, equidistantly spaced in 4.5 cm X 10.5 cm rectangle) chosen in each cerebral hemisphere prior to the application of external magnetic field (**Figure 1**).

Figure 1. The 32 matrix points and the reference points from the international 10-20-electrode placement system.

The recorded MEG activity from each point was obtained for 32 consecutive epochs of 1000 ms duration for each epoch (**3**). The MEG records were digitized with sampling frequency of 250 Hz and stored in a PC computer for off-line Fourier statistical analysis. Using Fourier statistical analysis it was obtained the spatial distribution of the MEG power spectrum amplitude for specified band frequencies for each measured brain point. These were expressed with the use of computer generated graphics in terms of the total average of equal (ISO) spectral amplitude (ISO-SA)

distribution of the surface of the scanned areas over the scalp utilizing the frequency bands of 2-7 Hz, 8-13 Hz, and 14-25 Hz (**3,20**). These maps were useful in obtaining clearly defined areas of high spectral density in the 2-7 Hz band frequency. In addition, the ISO-SA maps were helpful in providing clear identification of the coordinates of the point on the scalp where the MEG power spectrum emitted for the 2-7 Hz frequency has its maximal power as well as its maximal magnetic intensity. The isocontour lines corresponding to equal spectral amplitudes for each frequency band were calculated providing two-dimensional graphic representation. Different colors in these ISO-SA maps represent different spectral amplitudes (in fT/√Hz) for the same band frequency. In these graphic illustrations the large symbol"+" represents any one of the points (P3, P4, T3, T4, F3, and F4) as origin of the chosen reference system, depending from the recorded hemisphere. The recording reference system was based on the international 10-20 Electrode Placement system (**21**). In the same illustration, the white small "+" symbol represents the relative positions of the recorded 32 matrix points, in each hemisphere, with respect to the reference points for easy identification of the coordinates of the abnormal points.

Mapping the spectral power distribution over a brain surface, in the case where the MEG measurements are independently recorded for each position (as it was in the case where a single channel SQUID was used in the beginning), requires that the recorded MEG activity remains invariant in time (**3,4,19**). In order to insure that in the course of the MEG recordings the activity was not influenced by long-term variations, the recordings were repeated at various positions at different times.

This similarity is also evident in the ISO-SA maps seen in **Figure 2** which were obtained from an epileptic patient after measuring the same brain region in two different dates (**3,4,19,20**).

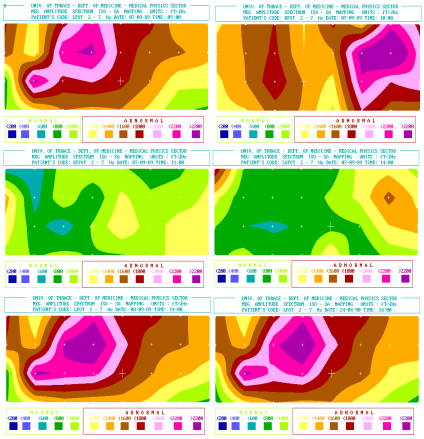


Figure 2. The ISO-SA maps of the left and right temporal, occipital and frontal brain regions respectively of an epileptic patient and also a comparison between the first measurement and that after two different dates for the left temporal region.

In terms of the MEG activity, it was proposed a functional definition of the presumed epileptic foci or abnormal brain region. A focus was defined as a circumscribed cerebral area where, in the band of 2-7 Hz MEG frequencies, it exhibited its maximal power spectral value and the densest concentration of ISO-SA contour lines (**3,19**). As a corollary to previously described functional definition of a focus or lesion (**3**), it was proposed that when a focus or lesion is identified in both brain hemispheres, the one which is characterized by the highest concentration of the ISO-SA lines in its morphology should be considered to be the dominant one. This method was considered to be simple since it represents pictorially the projected localization of maximal brain activity. By its nature (i.e., temporal and spatial averaging), the procedure eliminates short-term abnormal neuronal discharges in any cortical area, while it retains long lasting localized activation phenomena (i.e., random interictal abnormal neuronal activity). The main limitation of the method, however, was related to the fact that it relies on long data acquisition times resulting from the use of one channel SQUID.

The information obtained from each functional focal or lesion point regarding the emitted magnetic field intensity, frequency, and coordinates was subsequently stored in a special integrated circuit of an electronic device the principles of which are given in (**22**). This device consists of a generator of alternating low voltage, which can produce low frequencies from 2-7Hz (**Figures 3**).

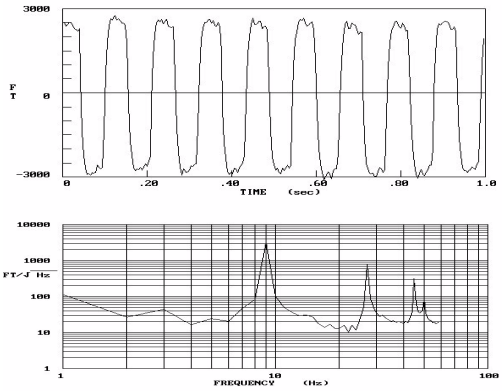


Figure 3.The waveform of the magnetic field emitted from a particular point of the electronic device and its power spectrum, which shows its emitted frequency.

This electronic device (**22**) was used to emit back magnetic fields of the same intensity and frequency to the previously defined focal or lesion points. **Figure 2** demonstrates the ISO-SA map of a epileptic patient before magnetic stimulation in which the maximal total average emitted power in the 2-7 Hz band frequency was >2200 fT/√Hz.
In **Figure 4** it is demonstrated the reduction of the emitted average power in the 2-7Hz band frequency of the same patient shown in previous **Figure 2**.

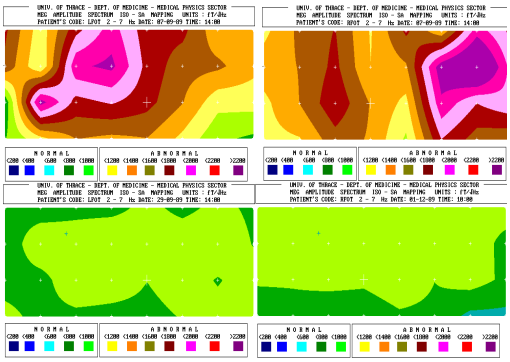


Figure 4. The ISO-SA maps of the same patient (Fig.2) before and after magnetic stimulation in which it is observed that the maximal total average emitted power in the 2-7 Hz band frequency is reduced to <1200 fT/√Hz.

The above-discussed method for measuring the brain dysfunctions in epileptic patients before and after the use of pTMS has been tested in more than 300 patients (**3,4,22**).

In the present study in order to substantiate more our findings we randomly choose 30 epileptic patients from among all who had pTMS for their treatment of seizures (**Table 1**).

Table 1. Classification of the examined patients according to their EEG diagnosis and response to pTMS. The results were of statistically significance ($p<0.02$, chi-square=4.8)

Response	Normal EEG	Abnormal EEG	Total
PR	4	6	10
FR	16	4	20
Total	20	10	30

The vast majority of the patients had focal epilepsy (93.1%) diagnosed on the basis of clinical observations and routine EEG recordings. All were receiving anticonvulsant medication at the time they receiving pTMS. Based on an independent chart review, the patients were divided into two groups according to the degree of anticonvulsant responsiveness to pTMS. The first group included patients who exhibited only partial anticonvulsant response (PR) to pTMS (i.e seizures re-curred within 12 months after pTMS and partial appearance of a-rhythm in their EEG by observing its amplitude spectrum in that frequency which is low). The second group included patients who demonstrated a favorable anticonvulsant response (FR) to pTMS (i.e seizure free for at least one year after pTMS and the appearance of a-rhythm in their EEG, in this case the amplitude spectrum in that frequency is very high). Thus, as it is seen in **table 1** 10 patients (33%) were classified as partial responders (PR) and the remaining twenty 20 (67%) exhibited a favorable anticonvulsant response (FR) to TMS.
From the partial responders to pTMS, normal EEG (i.e the appearance of low amplitude spectrum in the a-rhythm frequency) was seen only in 4 patients (40%). In contrast 16 out 20 patients (80%) who showed a favorable anticonvulsant response to pTMS had normal EEG (i.e the appearance of very high amplitude spectrum in the a-rhythm frequency) . This difference was found to be statistically significant ($p<0.02$,chi-square=4.8).
At this point it should be mentioned that the EEG and MEG diagnosis before and after pTMS is based on the appearance of a-rhythm amplitude in their power spectra amplitude distribution.

Figures 5,6 demonstrate the ISO-SA maps before and after magnetic stimulation for a PD patient in which also it is observed a reduction of the average emitted power.

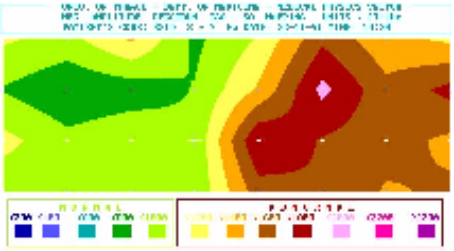


Figure 5. The ISO-SA maps of a PD patient before magnetic stimulation.

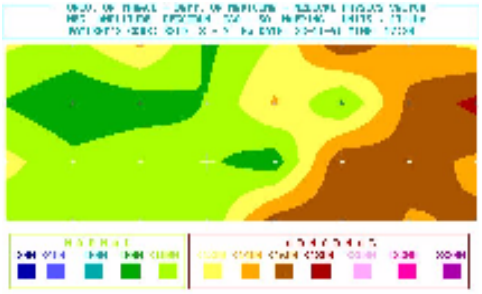


Figure 6. The ISO-SA maps of the same PD patient after magnetic stimulation.

The resultant 'cancellation' of the emitted power was observed after we applied the coils of the device for 1-2 minutes to the functional points of the patient's scalp. It is considered a focus or lesion to be 'cancelled' if the magnetic power emitted from the affected brain region had returned to a value of <1000fT/√Hz, which is a power value considered to be within normal limits.
The above method of pTMS was applied in the left-right temporal, frontal-occipital and vertex (2 minutes over each of the above regions) in each of the 32 matrix points (4.5X10.5 cm rectangle) of epileptic and Parkinson disease (PD) patients using the electronic device (**22**) and the emitted magnetic activity of these points of the brain was recorded again.
rapid attenuation of Parkinson disability and partial or complete resolution of the levodopa-induced dyskinesias, which is a common side effect complication of chronic dopaminergic therapy (**24,25**). Although the striking beneficial effects of the application of the pTMS on the clinical picture of the PD patients are well observed, the mode of action of pTMS in PD remains an open question.

The Multi-Channel MEG evaluation of PD patients before and after pTMS

Thirty more PD patients (22 males, 8 females; mean age 65 years, range 49-80 years) were referred to our laboratory by practicing neurologists. All patients had diagnosed independently to suffer from idiopathic PD. None of the patients has had a history of neurological disease other than Parkinson's disease. All patients had normal routine serum biochemical studies, as well as normal CT or MRI scans. In all cases informed consent for the methodology and the aim

The applied pTMS was adjusted to have similar field characteristics (magnetic field 1- 7.5 pT and frequency the α- rhythm of each individual patient (8-13 Hz)) as was recorded from the same patient's brain prior to the application of the pTMS. The time between the 1st MEG and the post-stimulation MEG is about an hour. In all patients placebo tests were also performed. None of the patients experienced side effects during or after the procedure. The above methods for measuring the brain MEG activity in PD patients before and after the use of external magnetic stimulation (**3,4,23**) have been tested in 30 patients aged 47 to 86 years (mean=69.1, SD=9.8).
The application of pTMS on the brain of the above PD patients was resulted in a

of the study was obtained from all patients prior to the procedure.
MEG measurements were performed in all PD patients using a whole-head Neuromag 122 SQUID system in a magnetically shielded room of low magnetic noise (**26,27**). The sampling frequency was 256Hz and digitized using a 12 bit precision analog to digital converter and filtered with cut-off frequencies between 0.3 and 40Hz.

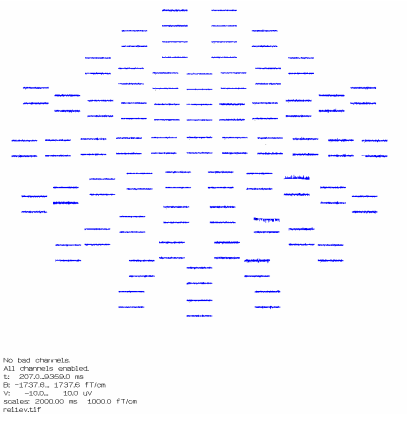
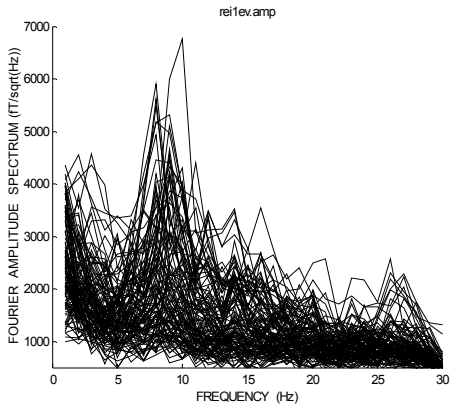


Figure 7.The MEG raw data recorded from a PD patient



Thus, this **Figure 7** illustrates the spontaneous MEG activities recorded from the 122 measured points from a PD patient using the 122 Channel SQUID, whereas **Figure 8** illustrates the Fourier power spectra amplitudes of the raw data presented in **Figure 8**.

The following **Figure 9** illustrates for the same PD patient the distributions for the first dominant frequencies corresponded to the first maximum amplitude of the MEG Fourier power spectrum obtained from each of the 122 measured brain points with the SQUID prior to the application of pTMS.

Figure 8.The power spectrum of the raw data of Figure 7.

wave signals with a specific frequency which is regulated by one integrated circuit which receives the frequency value from a keyboard or from a computer through a serial port and an interface integrated circuit and supplies a great number of coils, up to 122, with a proper spatial hemispheric arrangement to coincide with that of the 122 brain points measured by the SQUID. The time between the first recorded MEG and the MEG obtained after the application of the pTMS is about 24 hours. In the above patients placebo test were also performed, as it was done also with the previous

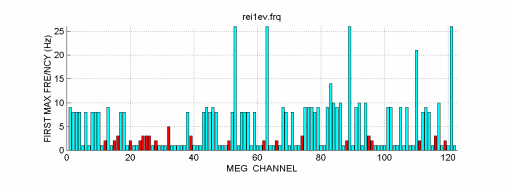


Figure 9. The distribution for the first dominant frequencies corresponded to the first maximum amplitude of the MEG Fourier power spectrum for each of the 122 measured brain points before the application of pTMS.

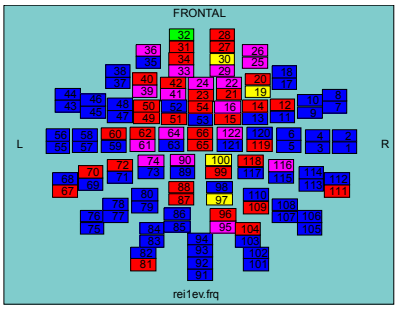


Figure 10. The spatial distribution for the first dominant frequencies of each of the 122 measured brain points prior to the application of pTMS. (red= 2 Hz; pink= 3Hz, yellow=4Hz, green= 5 Hz, blue>=6 Hz)

Finally, the above **Figure 10** illustrates the spatial distributions of the first dominant frequencies for each of the 122 measured points prior to the application of pTMS.

In all of the above PD patients, pTMS was applied with proper field characteristics (Intensity: 1-7.5 pT,; frequency : the α- rhythm of the patient: 8-13 Hz) with the ones obtained prior to pTMS using an electronic device (**28**). This device consists of an alternating current generator to produce square method using the one channel SQUID, without energizing the device, in order to evaluate the influence of pTMS. None of the patients experienced side effects during or after the procedure.The classification of these PD patients was examined according to their EEG and MEG diagnosis and their response to magnetic stimulation (**Table 2**).

Table 2. Classification of the examined patients according to their EEG diagnosis and response to

magnetic stimulation. This difference was found to be statistically significant (p<0.1,chi-square=2.55).

In this study the PD patients were divided into two groups according to the degree of their responsiveness to pTMS.

As it is seen in **Table 2**, 12 patients (40%) were classified as partial responders (PR) and the remaining 18 (60%) exhibited a favourable response (FR) to pTMS.
As is summarized in **Table 2** , from the PR to pTMS, normal EEG (i.e., the appearance of high amplitude of power spectrum in the α-rhythm frequency was seen only in 5 patients (41.67%). In contrast, 16 out of 18 patients (88.88%) who showed a FR to pTMS had normal EEG (i.e., the appearance of very high amplitude power spectrum in the α-rhythm frequency). This difference was found to be statistically significant (p<0.1,chi-square=2.55).
The results are also illustrated in **Figure 11** in which it is observed the distribution of the first dominant frequencies corresponded to the first maximum amplitude of the MEG Fourier power spectrum obtained from each of the 122 measured brain points with the SQUID for the same patient after the application of pTMS.

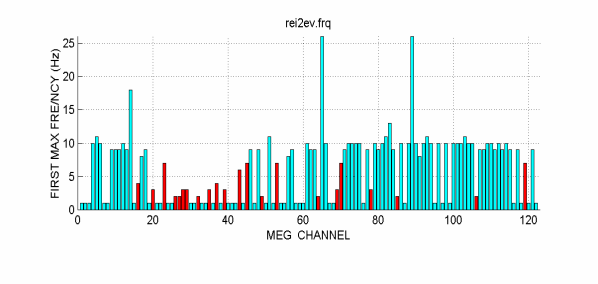


Figure 11. The distribution for the first dominant frequencies after pTMS.

Finally, **Figure 12** illustrates the spatial distributions for the first dominant frequencies of **Figure 11** in which it can be seen that most of the frequencies are increased with the application of pTMS (**29**).

Response	Normal EEG	Abnormal EEG	Total
PR	5	7	12
FR	16	2	18
TOTAL	21	9	30

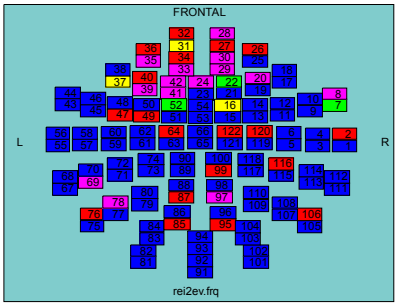


Figure 12. The spatial distribution for the first dominant frequencies after pTMS. (red=2 Hz; pink=3Hz, yellow=4Hz, green=5 Hz, blue>=6 Hz)

The different colors represent the different first dominant frequencies.

3. Discussion

Wide knowledge of the MEG techniques has been gained since the discovery of SQUID's. Regardless of the particular mix of technologies that have been used to image human brain function, the field of biomagnetism provides useful and invaluable information for future research, many of us in the field have repeatedly discovered to our amazement and delight. The use of the MEG techniques with the application of pTMS is a powerful combination to help us understanding and care of people who have CNS disorders. Researchers have begun to use MEG functional brain imaging in order to learn more about brain disturbances that afflict patients suffered from Parkinson and other brain disorders. However, the use of MEG in order to evaluate the human brain disturbances with the application of pTMS are not many in the field except those who have worked with the authors (**3,25**) and are continuing their work without using the powerful tool of SQUID technology (**24**).
Future research has to be addressed to some important issues concerning the use of MEG techniques with the application of pTMS. Thus, more clinical studies must be further investigated and alternative evaluation and detection methods must be developed.

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Magnetoencephalographic Findings in 2 Cases of Juvenile Myoclonous Epilepsy

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*Abstract.*PURPOSE: To find out the electromagnetic sources of epileptic activity in 2 patients with juvenile myoclonous epilepsy. MATERIAL AND METHODS: Patient 1 was a 22-y old female who experienced juvenile myoclonous epilepsy when she was 17 years old, whereas patient 2 was a 29-y old male and experienced juvenile myoclonous epilepsy when he was at the age of 18 years old.. Magnetoencephalography (MEG) was recorded with a 122-channel, whole-head system. Equivalent current dipoles (ECDs) were calculated for epileptic spikes on MEG recordings according to the single dipole model. RESULTS: Patient 1 had ECDs located at the stem and cerebellum, whereas her EEG spikes were located at the frontal area extended up to the occipital region. Patient 2 had ECDs located at the cerebellum, whereas his EEG spikes where located at the frontal area extended up to the occipital region also. CONCLUSIONS: In this study we localized functional disorders at the occipital areas of the brain. These results are in agreement with recent studies of idiopathic generalized epilepsy with quantitative MRI and Proton Magnetic Resonance Spectroscopy (1H-MRS). The validity of MEG in localizing the origin of juvenile myoclonus epilepsy might also be confirmed with larger studies.

Keywords: MEG; juvenile myoclonous epilepsy, ECD

1. Introduction

Juvenile myoclonic epilepsy (JME) represents the 5-11% of all epilepsy cases. It is a syndrome of idiopathic generalized epilepsy with an age-related onset of seizures; it is characterized by myoclonic jerks, tonic–clonic seizures and less frequently by typical absences. A normal recording was obtained on the initial EEG of 41% of patients, and 73.2% had at least one normal EEG recording during work-up. Serial EEGs seem to be fundamental to confirm diagnosis: 3 recordings identified 84% of cases, and this figure increases to 94% when 4 to 8 recordings are obtained, but there is no significant increase with more than 8 recordings. A correct diagnosis of JME has an important impact on treatment and outcome of patients. Delay in disease identification obstructs seizure control and may lead to status epilepticus, nonreversible brain damage, social difficulties, and even death (1). The typical ictal EEG of a myoclonic seizure displays the pattern of polyspike – and - wave, which consists of a group of 5 to 20 generalized, almost always symmetrical and high frequency spikes, usually followed by slow waves (2). The onset and maximal voltage are seen in the fronto-central regions, then spreading to the parietal, temporal, and occipital regions (2). This finding may be present in 22.2% to 51.4% of patients (3, 4). Magnetoencephalography (MEG) is a technique for measuring the magnetic fields associated mainly with intracellular currents, while the EEG measures mainly extracellular field potentials. Intracellular currents are well modeled by the "equivalent current-dipole" (ECD) model. This model allows characterization of the source of neuronal activity in the brain and specifically its position and strength. This is particularly useful in focal epilepsies, in which small areas of brain tissue trigger the epileptic activity and are important in obtaining a good spatio-temporal localization of the "foci". However, MEG might be helpful in more complex epileptic patterns (generalized epilepsy) in characterizing the early start of interictal and ictal activity. EEG can detect dipoles with any orientation, but is attenuated and distorted by various brain conductivities, skull, scalp and fluids: structures that only weakly affect the magnetic field. MEG is more sensitive than EEG to sources whose current flows are tangential to the scalp surface, but can only minimally detect current flows that are perpendicular to the scalp. Therefore MEG and EEG alone may only partly depict neuronal activities, but in combination produce a full image of the brain activity (5).

In this paper we describe MEG findings of 2 cases with JME with the location of the epileptic foci at the stem and cerebellum areas.

2. Patients and Methods

The patient 1 is a 22-y old female who experienced JME when she was 17 years old. Her EEG showed widely sharply slow theta waves at both sides of the frontal area with extension up to the occipital region. She experienced clusters of peak-waves at 2.5 Hz at both sides of the frontal area after the end of hyperpnoea. She had 2 to 3 incidents with loss of consciousness without spasms. During the first 2 episodes she was pale and abstracted for some seconds without fall. At the 3rd episode she fell down. She had anxious sleep with a lot of myoclonic jerks and quite often she loss urine. She was under therapeutic treatment with Depakin (1x3). She experienced epileptic seizures every 1-2 months. She also appeared symptoms of depression. Her EEG was improved. Her last seizure was 1 year ago.

The patient 2 is a 29-y old male and experienced JME when he was 18 years old, at the time of awaken. His EEG showed peak-wave discharges at the frequencies of 3.5-4 Hz with superiority at the anterior area. He experienced a great improvement after therapeutic treatment with Depakin (500, 1x2). His EEG was normal. There is now a reduction using the doses of Depakin.

The MEG recordings were carried out in a magnetically shielded room with a whole head 122-channel biomagnetometer (Neuromag-122, Helsinki, Finland) (6-8). The device employs planar gradiometers, which record at each of the 61 measurement sites, the magnetic field component normal to the helmet-shaped Dewar bottom surface. During the recordings the subject was sitting in a chair with his/her head covered by the helmet-shaped dewar. The MEG sampling frequency was 256 Hz and the associated Nyquist frequency was 128 Hz, which was well above of constituent frequency components of interest in our MEG recordings and avoiding aliasing artefacts. The time taken for each MEG recording was 3min. For each MEG spike, we calculated the single ECD source at the spike peak. The location, orientation, and strength of each ECD were calculated by using a single-sphere model of the skull available at the MEG workstation. We defined acceptable ECDs as those with a goodness-of-fit to the model of >70% and with ECD strength between 100 and 400 nAm (nano Ampere metre). Three fiducial points were defined on each patient's head surface. They were clear anatomic landmarks, the 2 preauricular points and the nasion. These 3 points define the coordinate system that includes the brain and the position of the magnetometers relative to it. The line between the preauricular points defines the x-axis of the coordinate system, with the positive direction being to the right. The line between the nasion and the mid-point of the x-axis and perpendicular to it, defines the y-axis and the line perpendicular to the x-y plane, passing through the intersection of the x and y-axes, defines the z-axis of the coordinate system. The positive y-axis passed through the nasion and the z-axis pointed upwards. The locations of the ECDs are estimated and projected onto the structural images of the brain (MRI), which displays the activated brain regions. A separate 18-channel EEG recording was also performed, according to international guidelines. Electrodes were placed using the 10–20 International System with bipolar and referential montages. Each recording lasted 30 min.

3. Results

The ECDs were calculated at spike peaks with a spherical model using a dipole-fit software available in our Lab. Waveforms were inspected preliminarily to eliminate possible artefacts. For the patient 1, a total of 24 ECDs of epileptic activity were observed: From the 24, the 21 ECDs were ascribed to a single epileptogenic focus at the stem and cerebellum area, whereas the remaining 3 did not provide reliable localization (Figure 1). For the patient 2, a total of 199 ECDs of epileptic activity were observed which were localized at a single epileptogenic focus at the cerebellum area (Figure 2). The EEGs exhibited epileptic activity at the frontal area extended to the occipital areas for both patients. Their MRIs were normal.

4. Discussion

There are not enough data for the investigation of JME with MEG in the international literature. Patients with progressive myoclonic epilepsy have been studied by MEG and some researchers suggest that MEG allows increased spatial resolution and a more precise localization of premyoclonic spikes (9). In our study, the differences in MEG and EEG findings are due to the following reasons: greater sensitivity of MEG in the different conductivities; superiority of MEG channels; MEG localizes the

epileptic activity more precisely at the frontal-occipital direction whereas the EEG at the left-right temporal direction. MEG and EEG findings coincide for the localization of epileptic foci in 35%. The MEG has more accurate localization of epileptic activity in 40%. For patient 1, the origin of these spikes was localized in 21 cases out of 24 in the same small volume of the single epileptogenic focus at the stem and cerebellum area, thus giving a strong indication that this area is responsible for interictal epileptic activity. For patient 2 the epileptic activity was localized at a single epileptogenic focus at the cerebellum area.

Visual assessment of routine MRI in patients with JME is normal (10). However, recently developed neuroimaging techniques have detected structural abnormalities. An increase in cortical gray matter was identified in mesial frontal lobes (11), frontobasal regions, and anterior portion of the thalamus (12). Additionally, 40% of individual patients with JME, a syndrome of idiopathic generalized epilepsy in adolescence, had significant abnormalities of cerebral structure (12). Magnetic resonance spectroscopy found a reduced N-acetyl aspartate in the temporal lobe (11). Woermann et al (13) applied the automated and objective technique of statistical parametric mapping to the analysis of structural MRI from 20 patients with JME and 30 control subjects. The voxel-based of statistical parametric mapping comparison between the group of JME patients and the control subjects showed an increase in cortical grey matter in the mesial frontal lobes of the patients. Analysis of individual patients revealed significant abnormalities of cortical grey matter in 5 out of 20 JME patients, 4 of whom had been shown to have widespread abnormalities. Simister et al. (14) reported a study of GABA plus homocarrnosine and glutamate plus glutamine concentrations in the occipital lobes in 15 patients with idiopathic generalized epilepsy using proton magnetic resonance spectroscopy (1H-MRS). Grey-matter proportion, glutamate plus glutamine and GABA plus homocarrnosine were all elevated in the occipital lobes.

1H-MRS provides information on certain brain metabolites. As N-acetyl aspartate is thought to be a neuronal marker, a reduction in its level can be an indication of neuronal damage or dysfunction. Both prefrontal and thalamic N-acetyl aspartate concentrations were found to be significantly lower in idiopathic generalized epilepsy patients than in controls. A negative correlation was found between N-acetyl aspartate levels and duration of epilepsy, indicating that thalamic dysfunction may be progressive. 1H-MRS has revealed increased levels of glutamate plus glutamine in frontal lobes and elevated levels of glutamate plus glutamine and GABA in the occipital lobes of patients with idiopathic generalized epilepsy. Disorders of GABA and glutamate regulation are closely linked with seizure disorders (14,15). The combination of neuroimaging techniques, provide us the potentiality to have 3-D structural and functional brain maps, which allow us to identify accurately the epileptic regions of the brain.

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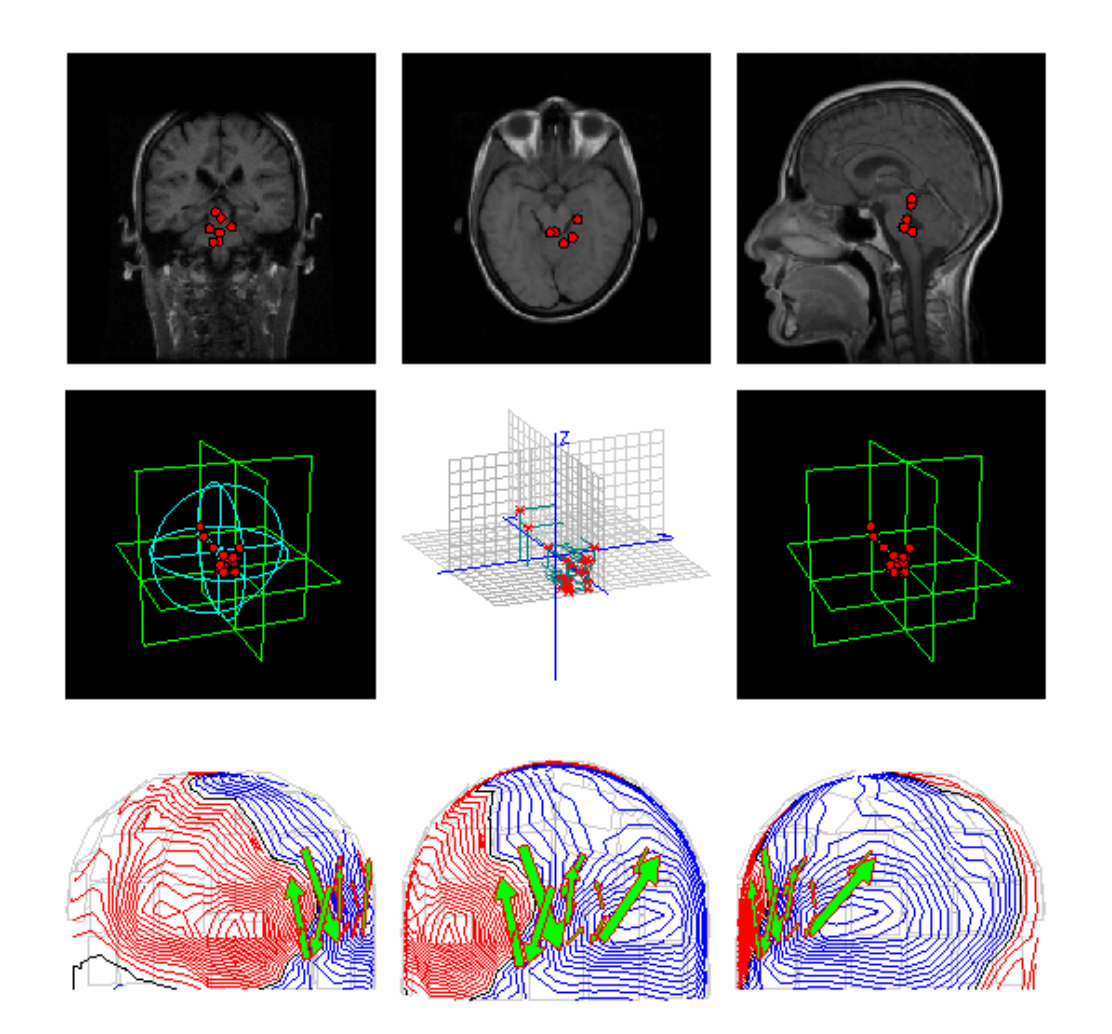

Figure 1. A) Coronal, sagittal and axial MRI images for the patient 1 with the superposition of the active ECDs. B) The whole number of dipoles in the x, y, z axes. C) The scalp ISO-field distribution and the ECD dipoles indicated by the arrows

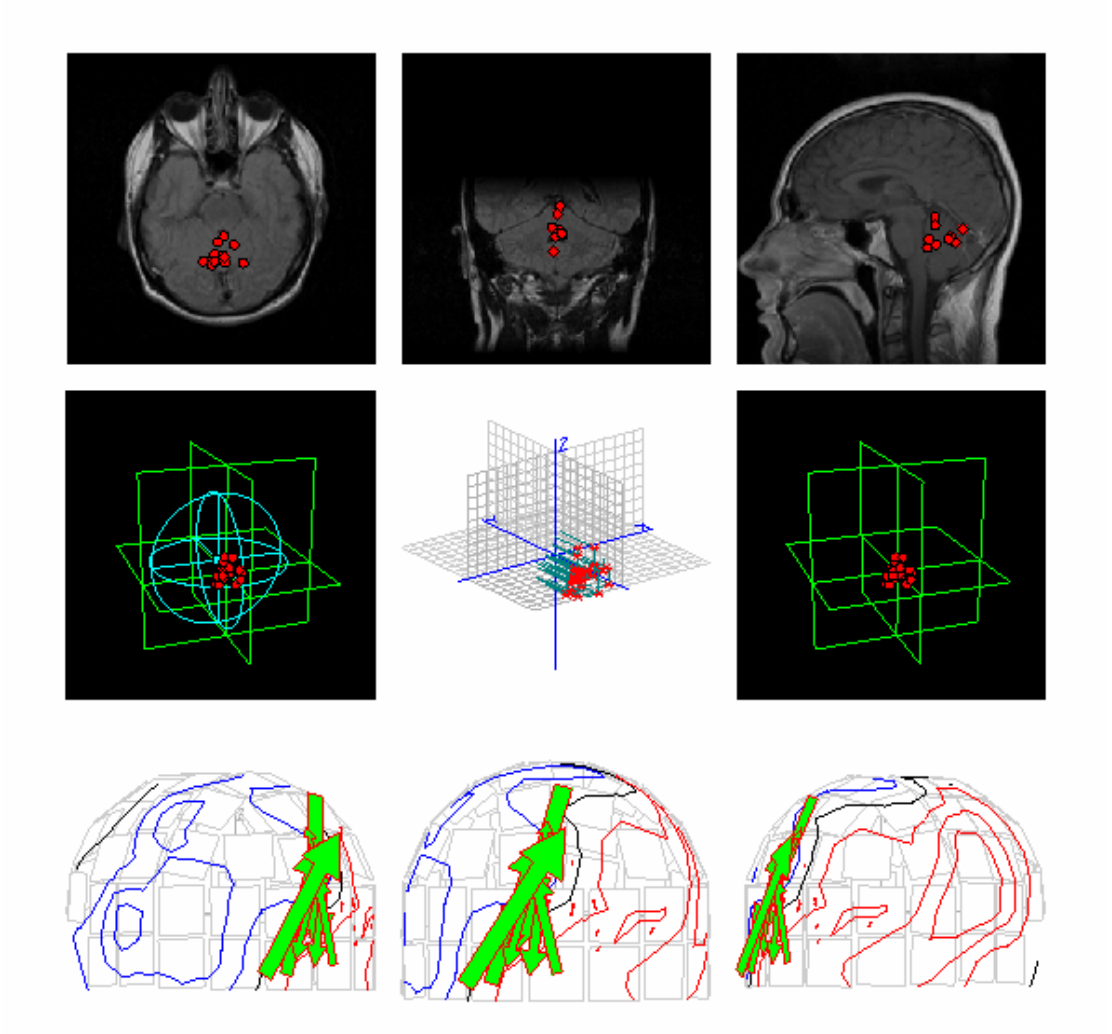

Figure 2. A) Coronal, sagittal and axial MRI images for the patient 2 with the superposition of the active ECDs. B) The whole number of dipoles in the x, y, z axes. C) The scalp ISO-field distribution and the ECD dipoles indicated by the arrows

Magnetic Stimulation can Modulate Seizures in Epileptic Patients

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Abstract: The aim of this study is to investigate the influence of external magnetic stimulation (EMS) in epileptic patients using magnetoencephalographic (MEG) measurements and non-linear analytic techniques. The examined group consisted of 15 men aged 19-56 years (mean: 39.5±11.3) and 15 women aged 15-53 years (mean: 36.7±11.4). For each one the magnetic activity was recorded from 32 points for each temporal lobe. External magnetic stimulation (EMS) with proper field characteristics (intensity: 1-7.5 pT, frequency: the **α**-rhythm of the patient (8-13Hz)) was applied in the frontal, occipital and temporal lobes for 2 to 6 minutes and the emitted brain magnetic activity was recorded again. In order to investigate if there is any alteration in the MEG complexity underlying the neural dynamics characterizing the pathologic brain before and after the EMS, chaotic analysis approach was applied for the estimation of the dimensional analysis of the existing strange attractors. The application of EMS resulted in rapid attenuation of the MEG activity of epileptic patients. The obtained results of the dimensionality calculation provide a shift from lower to higher dimensional values. Such a shift is an indication that we are dealing with a chaotic system similar with the one characterizing normal subjects. The increased values of the dimensional complexity and the lower activity of the MEG after the application of EMS strongly supports the beneficial effects of EMS in epileptic patients.

Key words: MEG; Magnetic stimulation; Epilepsy; Non-linear analysis.

1. Introduction

The cerebral cortex is known to produce weak magnetic fields that can be recorded using the Superconducting Quantum Interference Device (SQUID) [1]. Such measurements are known as magnetoencephalogram (MEG). Unlike the electroencephalogram (EEG), the MEG is not subject to interferences from the tissues and fluids lying between the cortex and the scalp. The magnetic fields produced by the cerebral ion movements are about one hundred million times smaller than the earth's magnetic field [1,2]. Ionic movements throughout the neuronal cell body creating a current dipole follow changes in membrane potential. The orientation of the current dipole is a critical factor, which affects the measurement of magnetic fields. The MEG produced by such fields is exclusively created by a flow of electric currents tangential to the skull surface and therefore the signal will originate maximally from the cerebral sulci (where the pyramidal cells are more favorably oriented) and only minimally from the gyri surface where their orientation is less favorable [1]. As it was pointed out by Elger et al. [3] the single dipole model is not the most appropriate model for the conceptualization of seizure activity since: a) an epileptic focus generates different types of seizure activity; b) the brain area which generates an epileptic discharge varies and different neuronal populations may contribute to a single epileptic event; c) the synchronized potentials of "epileptic" neurons give rise to synchronized projected synaptic activity; d) the interictal activity may be localized only in a limited number of patients with seizure disorders. In order to avoid these difficulties we have proposed [4] an alternative approach for the evaluation
of the MEG recorded from patients with CNS disorders. Thus, instead of studying the surface distribution of the MEG in the time domain our method was based on investigating the surface distribution of the recorded MEG activity in the frequency domain. This was proposed on the basis that the surface distribution of the spectral energy
would exhibit patterns for specified locations of CNS disorders [3]. The MEG information obtained from each measured point of patients' brain activity regarded the emitted magnetic field intensity, frequency and coordinates was subsequently stored in a special integrated circuit which was subsequently used to

energize an electronic device the principles of which have been previously published [4-9]. The latter was properly used to apply external magnetic stimulation (EMS) to epileptic patients with magnetic fields of certain characteristics (intensity: 1-7.5 pT, frequency: the **α**-rhythm of the patient (8-13 Hz)). To achieve this, the coils of the magnetic stimulator were applied for 1-2 min to each point on the patients' scalp. The application of EMS was justified in view of the observation in animals and humans which showed that administration of low frequency magnetic fields altered the electrical activity in the brain and thus influenced the frequency or severity of seizures [10]. This application of external magnetic stimulation convinced more and more scientists that it will be a new revolutionary approach toward treatment of various neural pathologies because it is safe, non-invasive and easy to apply. On the other hand according to the theory of non-linear dynamical systems and chaos [11,12] the dynamics of any biological system can be quantified and described by means of some concepts and parameters, such as the strange or chaotic attractor, the correlation dimension of the reconstructed phase space, the Lyapunov exponents
and so on. These concepts reflect some geometrical properties of the reconstructed phase space of the dynamical system under consideration and it can be extracted. Of vital importance in the chaotic analysis of a dynamical system is the evidence for the existence of low dimension chaotic attractors and the estimation of the correlation dimension D of the attractor.
The aim of this study is to investigate any alteration in the brain dynamics of epileptic patients after the application of external magnetic stimulation (EMS) using MEG measurements and non-linear analytic techniques.

2. Materials and Methods

The epileptic patients were referred to our Laboratory by practicing neurologists. They consist of 15 men aged 19-56 years (mean = 39.5, SD=11.3) and 15 women aged 15-53 years (mean = 36.7, SD=11.4). All patients have been diagnosed independently to suffer from idiopathic epilepsy and had normal routine serum biochemical studies, as well as, normal CT or MRI scans. Due to the limited resolution and low sensitivity of the MEG methods we have chosen to use EEG in epileptic patients for its important implications for clinicians which don't have access to the very expensive MEG systems in order to use EMS. As we have seen, a number of them appeared to have normal EEG, although the patients experienced seizures. The Hospital Ethics Committee approved the whole examination procedure and informed consent for the methodology of the study was obtained from all patients. Biomagnetic measurements were performed using a second order gradiometer SQUID (model 601 of the Biomagnetic Technologies Inc.), which was located in an electrically shielded room of low magnetic noise. The MEG recordings were performed after positioning the SQUID sensor 3 mm above the scalp of each patient using a reference system. This system is based on the International 10-20 Electrode Placement System (Jasper 1958) and uses any one of the standard EEG recording positions as its origin (in this study we used the P3, P4, T3, T4, F3, and F4 recording positions) [4-9]. Around the origin (T3 or T4 for temporal lobes) a rectangular 32-point matrix was used (4 rows × 8 columns, equidistantly spaced in a 4.5 cm × 10.5 cm rectangle) for positioning of the SQUID [4-9]. The MEG was recorded from each temporal lobe at each of the 32 matrix points of the scalp for 32 sec and was digitized with a sampling frequency of 250 Hz (frequency resolution of the power spectrum being 1 Hz). The MEG signal was band-pass filtered with cut-off frequencies of 0.1 and 60 Hz.
The MEG recordings were digitized using a 12 bit precision analog to digital converter with a sampling frequency of 250 Hz, and were stored in a PC peripheral memory for off-line Fourier statistical analysis. The method, by its nature (i.e. temporal and spatial averaging), eliminates short-term abnormal artifacts in any cortical area, while it retains long lasting localized activation phenomena. External magnetic stimulation (EMS) was applied in the frontal, occipital and temporal lobes using an electronic device [4-9] and the emitted magnetic activity was recorded again. The electronic device consists of a low voltage generator, which can produce low frequencies from 2-13 Hz to a group of coils of 1 cm diameter. The 32 coils are enclosed between 2 parallel plane surfaces with the axes of the coils perpendicular to these surfaces. They are situated on the 32-point matrix, which is defined previously. The applied EMS carried similar field characteristics (intensity: 1-7.5 pT and frequency the **α**-rhythm of the patient (8-13 Hz)), the latter recorded from the patients. The time between the 1st MEG and post-stimulation MEG is about an hour. None of the patients experienced side effects during or after the procedure.

2.1 Chaotic Analysis of the MEG Data

We applied non-linear analysis in order to investigate the difference in the complexity underlying the dynamics characterizing the brain activity of the epileptic patients before and after EMS. According to Grassberger and Procaccia [13,14], the dynamics of the system can be experimentally reconstructed from the observed MEG time series Bi=B(ti) (i=1,2,...,N) and the vector construction of Vi is given by the following equation:

$$Vi=\{Bi,Bi+\tau,...,Bi+(m-1)\tau\} \quad (1)$$

This equation gives a smooth embedding of the dynamics in an m-dimensional phase space. The evolution of the system in the phase space – once transients die out – settles on a submanifold, which is a fractal set called the strange attractor. The strange attractor can be described by a geometrical parameter: the correlation or fractal dimension (D). This parameter is related to the number of variables required to define the attractor within the phase space and it can be estimated from an experimental time series by means of the correlation integrals C(r,m) defined as follows:

$$C(r, m)=\lim_{n\to\infty}(n(n-1)/2)^{-1}\sum_{i=1}^{n-1}\sum_{\substack{j=1+i \\ i\neq j}}^{n}\Theta(r-|V_i-V_j|) \quad (2)$$

where θ (u) is the Heaviside function defined as $\theta(u)=1$ for $u>0$ and $\theta(u)=0$ for $u\leq 0$);mis the embedding dimension; n is the number of vectors constructed from a time series with N samples, and is given by the formula $n=N-(m-1)\tau$ (where τ is a delay parameter which is equal to the first zero crossing of the autocorrelation time of the MEG signal); and B is a correction factor for spurious influences of autocorrelation [15]. The correlation dimension of the attracting submanifold in the reconstruction phase space is given by

$$D=\lim_{\substack{r\to 0 \\ m\to\infty}}\partial(\ln C(r,m))/\partial(\ln(r)) \quad (3)$$

In the case of a chaotic signal exhibiting a strange attractor, there is a saturation value (plateau) in the graph of the slope of the function D, equal to ln(r(2–r)). This value remains constant, although the signal is embedded in successively higher-dimensioned phase spaces.

If the dimensionality of the strange attractor is shifted to higher values (approaching in this way the dimension of the MEG from a normal subject), then the characteristics of such MEG data are continuously used as new inputs to the magnetic stimulator applied to the patient's brain. Recording the MEG activity over the scalp in the case where the measurements are independent for each position requires that the MEG activity remains invariant in time. In order to ensure the stability of MEG measurements we repeated the recordings at various
positions at different times and found that there was very little difference in the measurements as much as 60 min apart during the experiments because there was a constancy in the D values.

3. Results

The above-discussed method for measuring the brain dysfunctions in epileptic patients before and after the use of EMS has been tested in over 300 patients. In the present study in order to substantiate more our findings we randomly choose 30 epileptic patients from among all who had EMS for their treatment of seizures (table 1). The vast majority of the patients had focal epilepsy (93.1%) diagnosed on the basis of clinical observations and routine EEG recordings. All were receiving anticonvulsant medication at the time they receiving EMS. Based on an independent chart review, the patients were divided into two groups according to the degree of anticonvulsant responsiveness to EMS. The first group included patients who exhibited only partial anticonvulsant response (PR) to EMS (i.e seizures recurred within 12 months after EMS and partial appearance of **α**-rhythm in their EEG as denoted by low amplitude). The second group included patients who demonstrated a favorable anticonvulsant response (FR) to EMS(i.e seizure free for at least one year after EMS and the appearance of **α**-rhythm in their EEG, in this case with high amplitude (table 1). Thus, 10 patients (33%) were classified as partial responders (PR) and the remaining 20 (67%) exhibited a favorable anticonvulsant response (FR) to EMS. From the partial responders to EMS, normal EEG (i.e., the appearance of high amplitude spectrum in the **α**-rhythm frequency) was seen only in 4 patients (40%). In contrast 16 out 20 patients (80%) who showed a favorable anticonvulsant response to EMS had normal EEG (i.e., the appearance of very high amplitude spectrum in the **α**-rhythm frequency). This difference was found to be statistically significant ($p<0.02$, chi-square=4.8). At this point it should be mentioned that the EEG

and MEG diagnosis before and after EMS is based on the appearance of **α**-rhythm amplitude in their power spectra amplitude distribution. The effects of EMS and non-linear analysis in one of the examined epileptic patients is now reported in detail, whereas the results of the 30 randomly selected patients are summarized in (table 1). Thus, the results discussed in the following referred to a 39-year old woman suffered from secondary generalized tonic-clonic seizures since the age of 12. She had been treated with various drugs and her current medication was carbamazepine (600 mg/d) and sodium valproate (2000 mg/d). Prior to her visit to our laboratory she experienced 8-10 convulsions daily occurring randomly during the day and night hours. A routine EEG and MEG were taken prior to magnetic stimulation which revealed very low amplitude alpha rhythm activity. The patient was followed up regularly for several years during which time we were applying EMS. The beneficial effects of the first EMS application lasted for 2-3 days and then it had to be repeated 3-4 times per week. Then she performed the application of magnetic fields at home with a portable electronic device (Ergo, Athens) consisting of a generator, which produces alternating weak magnetic fields. After approximately 5 months the beneficial effect become more permanent and the epileptic patient had to apply EMS less often. She continued to visit our lab twice a year in order to examine her improvement. She will discontinue the EMS application when her improvement becomes permanent.

Figs 1A,B show the MEG time series and the slopes of the correlation integrals, showing the correlation dimension of the strange attractor prior to the application of EMS. Figs 1C-F exhibit the MEG time series and the dimension correlation after the 1st EMS where the correlation dimension is progressively shifted to higher values, whereas figures 1E,F show the MEG time series and the dimension correlation after the 2nd EMS which are comparable to figures 1G,H which give MEG time series and the correlation dimension from normal subjects. In the 30 examined patients listed in table 1 the mean value of the correlation dimension before EMS was 6.27 (mean=6.27, SD=0.67) whereas after EMS was 11.06 (mean=11.06, SD=1.16).

Fig 1. A) MEG time series for 1 sec obtained from a brain point of the epileptic patient before magnetic stimulation. B) Plots of the slopes of the correlation integrals as a function of ln(r) for the MEG time series of fig 1A. C)MEG time series for 1 sec obtained for the same brain point of the epileptic patient after the first application of magnetic stimulation. D) Plots of the slopes of the correlation integrals as a function of ln(r) for the MEG time series of fig 1C. E)MEG time series for 1 sec obtained for the same brain point of the epileptic patient after the second application of magnetic stimulation. F) Plots of the slopes of the correlation integrals as a function of ln(r) for the MEG time series of fig 1E. G) MEG time series for 1 sec from a point of a normal subject for purpose of comparison. H) Plots of the slopes of the correlation integrals as a function of ln(r) for the MEG time series of fig 1G.

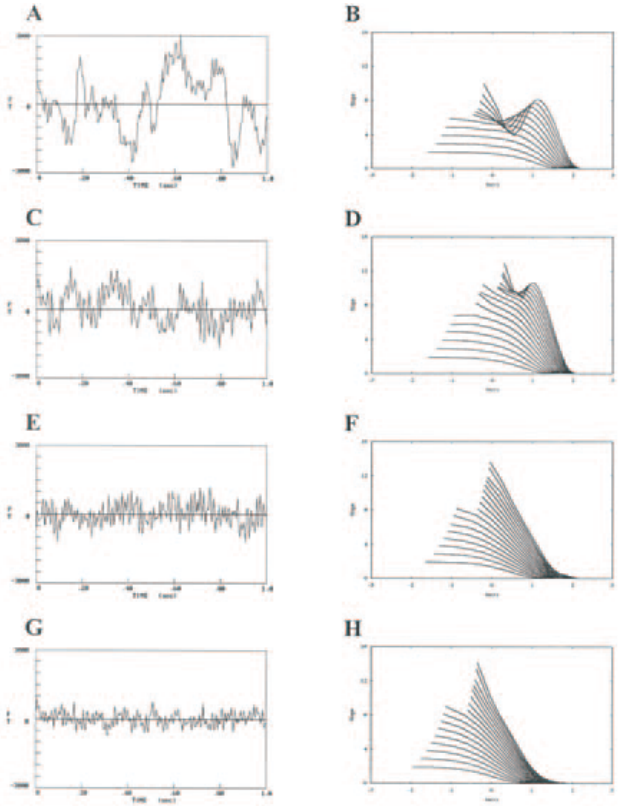

Table 1. The clinical data of the 30 patients and their response to EMS (N: normal; A: abnormal; PR: partial response; FR: favorable response; DIAGBMF: Diagnosis before magnetic field; DIAGAMF: Diagnosis after magnetic field.

Response	Normal EEG	Abnormal EEG	Total
PR	4	6	10

FR	16	4	20
Total	20	10	30

4. Discussion

The application of EMS has been used recently by several authors with transcranial [16,17] and intracranial [18,19] methodologies and it is proven to be a valuable tool for managing CNS disorders. In our patients we have observed a reduction of the average magnetic power of the MEG recordings after EMS as it was shown previously in [6] and also a clinical attenuation of patients' seizure activity as it was explained by [10].

The possible mechanisms by which magnetic fields have attenuated the patients' symptoms are still controversial. However, one possible electrophysiological explanation for the efficacy of magnetic stimulation has been provided by the proposed "neural net model" [7], which suggests that magnetic stimulation causes a temporary neuronal inhibition in regions exhibiting paroxysmal discharges. The hypothesis is in accordance with data presented by other investigators. However, it is known that magnetic fields alter the activity of the pineal gland, which has been shown to regulate dopaminergic, and endogenous opioid functions [20]. On a cellular level, the effects of magnetic fields on seizure activity may be related to alterations in properties and stability of
biological membranes and their transport characteristics including their intra- and extra cellular distributions and flux of calcium ions [10].

Another explanation for the management of epileptic activity using EMS is based on Morrell's hypothesis that every stimulus entering the brain is maintained for a certain period of time representing the short-term memory of the particular stimulus experience [21]. If the stimulus experience persisted for an extending period of time then the short-term memory of the presented
stimulus is converted to the permanent memory of the stimulus. Based on this principle from neurophysiology it may be possible to make the brains of epileptic patients convert their abnormal activities to normal using EMS of proper frequencies and intensities.

Furthermore, the application of dimensionality calculation of the MEG signals provides an additional criterion in comparing the correlation dimension of the MEG data obtained from the epileptic patients before and after EMS with normal subjects. Thus, comparing the existed values of the correlation dimension of the MEG obtained prior and after EMS with the corresponding dimension
from the MEG of a normal subject we can evaluate the relation between the functionality of the brain after EMS. Moreover, such evaluation can be achieved by comparing the estimated correlation dimension of the strange attractors underlying brain dynamics of subjects with three different conditions (abnormal, after EMS and normal), where it was observed a shift in the correlation dimension from lower to higher values. The lower correlation dimension corresponds to the epilepsy, which is characterized by reduction of the complexity of underlying neural dynamics [22].

The observed shift to higher values of the estimated correlation dimension reflects an increase in the complexity of the neural dynamics characterizing the brain dynamics of the epileptic patient under the influence of EMS. In terms of the pathophysiology of epilepsy, the increased complexity, which appeared in the MEG time series after EMS, can be expressed as a distortion of the high rhythmicity or abnormal synchronization and coherence of neural activity, which characterized brain activity of epileptic patients. Such a distortion is an indication that we are modulating seizure activity in such a way that the characteristics of the time series are
approaching the behavior of normal subjects.

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Nonlinear Analysis of Biomagnetic Signals Recorded from MALT Type Gastric Malignancy

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*Abstract.*Objective: To investigate the presence of any non-linearity in the biomagnetic recordings of low-grade gastric lymphoma of mucosa associated lymphoid tissue (MALT) type before and after surgery and in order to find the differences in the mechanisms underlying the gastric mucosa waves, we calculated the correlation dimension which is a measure of the complexity of the dynamic system.
Case report: A 47-years old female was referred to our department with a 2 years clinical history of a low-grade gastric lymphoma MALT type associated with Helicobacter pylori. The disease showed no histological remission after cure of H-pylori infection and subsequent treatment with chemotherapy (6 courses), and the patient was classified as stage IIEA. A decision for surgical resection was made. Biomagnetic activity was recorded in the target area before and after surgery.
Results: Applying the nonlinear analysis to the biomagnetic signals of the MALT type gastric lesion before and after surgery, we observed a clear saturation value for the lesional over the disease free tissue.
Conclusion: A comparison of the saturation value in the biomagnetic recordings before and after resection of lymphoma may be of help to evaluate the functional changes in the gastric mucosa dynamics.

Keywords: biomagnetometry, MALT lymphoma, gastric lymphoma, nonlinear analysis

1.Introduction

During recent years, several aspects of nonlinear dynamics have been explored in physics and medical engineering [1]. Mathematical concepts can be helpful in representing the biological world in various ways. Descriptions of linear and non-linear systems, chaos theory, fractal geometry and neural networks all find relevance to the study of oncological problems. The complexity of biological processes and the inter-relationships of many different factors, serve simplify with mechanical models the biological behavioral patterns, whether on the genetic, cell, tissue, organ, whole body or tumor scale. Dynamic mathematics describes systems that move or change in space and time. This is the nature of biological processes [2,3]. Cancer reflects dynamical and multistage processes. Oncogenes gain malignant function through mutation or chromosomal defect or through viral acquisition into the genome. Tumor suppressor genes promote neoplasia in consequence of the loss of their normal regulation. Chaos may thus manifest in tumors as a gain of complexity (tumor promoter genes) or as a loss of complexity (allelic loss, tumor suppression genes) [4].
There are no references about the application of nonlinear dynamic analysis in the biomagnetic activity of the gastric mucosa thus in this pilot study we measured the complexity of the gastric mucosa activity in MALT type lymphoma before and after surgery to ascertain whether nonlinear dynamics measured by dimensional complexity could be able to discriminate the lesional from normal tissue.

2.Case report

A 47-year-old woman was referred to our hospital with 2 years clinical history of a low grade MALT type gastric lymphoma not responded to helicobacter pylori eradication and subsequent chemotherapy administration (6 courses). The patient was classified as stage IIEA, based on physical examination, blood and biochemical parameters, intestinal radiological series, computed tomography scan, bone marrow biopsy, peripheral blood and bone marrow immunophenotype and HIV serology. A decision for surgical resection was made. Biomagnetic recordings from the gastric tissue (magnetogastrogram, MGG) were obtained by a single channel second order gradiometer DC-SQUID (MODEL 601;Biomagnetic Technologies Inc., San Diego, USA) before and after surgery [5-7]. Five points were selected for examination after positioning the SQUID sensor 3 mm above the target area. Point 5 was located at the very center of the MALT type lesion, whereas points 1-4 were located at the periphery of the examined area. The Hospital Ethics Committee approved the whole examination procedure.

2.1Theory and algorithm

Nonlinear analysis was applied for the estimation of the dimension of the strange attractor

characterizing the MGG time series obtained from the patient. According to Grassberger and Procaccia [8], the dynamics of the system can experimentally reconstructed from the observed MGG time series $B_i=B(t_i)$ (i=1,2...N) and the vector construction of V_i is given by the following equation:

$$V_i=\{B_i,B_{i+\tau},...,B_{i+(m-1)\tau}\} \quad (1)$$

This equation gives a smooth embedding of the dynamics in a m-dimensional phase space. The evolution of the system in the phase space, once transients die out, settles on a submanifold, which is a fractal set, the strange attractor. The strange attractor can be described by a geometrical parameter, the correlation of fractal dimension D. This parameter is related to the number of variables required to define the attractor within the phase space and it can be estimated from an experimental time series by means of the correlation integrals C(r,m) defined as follows:

$$C(r,m)=2/N^2 \sum_{i=1}^{N-B} \sum_{j=i+B}^{N} \Theta(r-|V_i-V_j|) \quad (2)$$

Where Θ (u) is the Heaviside function defined as (Θ(u)=1 for u>0 and Θ(u)=0 for u≤ 0), m is the embedding dimension and n is the number of vectors constructed from a time series with N samples, given by the formula $n=N-(m-1)\tau$ (Here τ is a delay parameter which is equal to the first zero crossing of the autocorrelation time of the MGG signal) and B is a correction factor for spurious influences of autocorrelation [9]. The correlation dimension D of the attracting submanifold in the reconstruction phase space is given by:

$$D=\lim_{\substack{r\to 0 \\ m\to\infty}} \partial(\ln C(r,m)) / \partial(\ln(r(r-2))) \quad (3)$$

In the case of a chaotic signal exhibiting a strange attractor, there is a saturation value (plateau), in the graph of the slopes D v's ln(r(2-r)). This value remains constant, although the signal is embedded in successively higher-dimensioned phase spaces. Using the above method the correlation dimension D of the selected MGG time series was estimated for the magnetic activity of the gastric mucosa harboring lymphoma before and after surgery.

3.Results

Fig 1A demonstrates the ln C(r) versus ln (r) plots from a biomagnetic signal recorded from the lymphoma tissue before resection.

Fig 1B shows the estimated correlation dimensions of the slopes of Fig.1A versus embedding dimensions before and after

resection. We observe a clear saturation value around 12 (mean=12.32±0.95 for the 5 points selected for the examination) whereas after surgery there is no saturation.

Fig 2 demonstrates the histological features of MALT type gastric lymphoma. The lymphomatous infiltrate around the follicles in the lamina propria, spreads out into surrounding tissue and invades gastric glands to form lymphoepithelial lesions. The tumor cells are small to medium sized with moderately abundant pale staining cytoplasm and nuclei with an irregular outline close resembling to the nuclei of centrocytes (small cleaved cells).

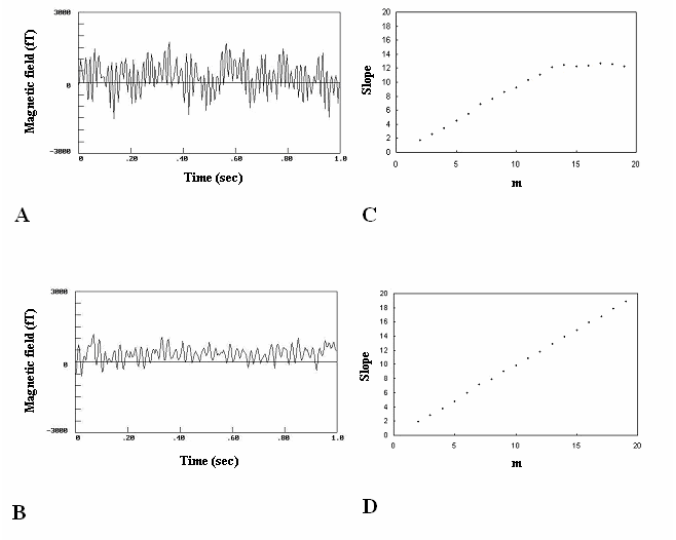

Fig 1. A) ln C (r) versus ln (r) curves for increasing embedding dimensions (2 to 20) B) Slopes versus embedding dimensions reveal that there is a saturation value around 12 before resection and absence of saturation after treatment.

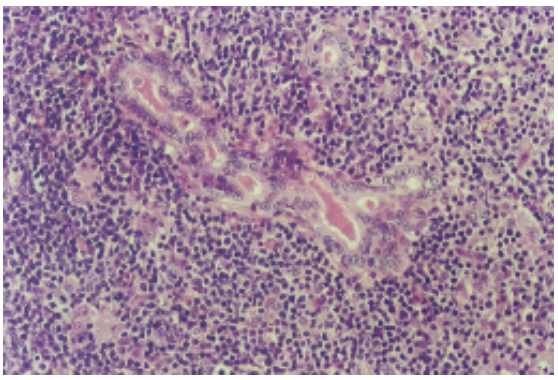

Fig 2. The histological features of MALT type gastric lymphoma

4.Discussion

According to the theory of nonlinear dynamical systems and chaos [10] the dynamics of any physical or biological system can be quantified and described by means of some new terms and concepts, such as the strange or chaotic attractor and the correlation dimension of the reconstructed phace space. These concepts reflect some geometrical properties of the reconstructed phase space of the dynamical system under consideration and it can be extracted. Of vital importance in the chaotic analysis of a dynamical system is the evidence for the existence of low dimension chaotic attractors and the estimation of the correlation dimension D of the attractor [11-15].

The difficulty in applying fractal and chaotic mathematical processes to tumor biology is clear from the paucity of useful models in clinical practice. Tumors are nevertheless unstable systems, as illustrated by their heterogeneity in tumor genetics, aneuploidy, morphology and growth patterns, for example. The change from cumulative minor genetic dysfunctions to catastrophic malignancy may thus have mathematical parallels. If chaotic signals do emanate from tumors, we may find them in the structural morphology of the whole tumor or its component parts, in the dynamic growth characteristics, in their patterns of behavior or even in their response to therapy. It is the instability associated with a tumor (volume increase, invasion or metastasis) rather than the existence of the tumor, which kills. The restoration of stability or steady state symbiosis of a tumor with its neighboring tissues may be an important objective in therapeutics.

Concerning differential diagnosis of the outline of a benign process could be compared with a malignant one by using the biomagnetic chaotic signals emitted by tumor tissue. We found that lymphoma tissue is characterized by lower chaotics. By comparing the correlation dimension it can be observed that there is a clear saturation value around 12 before treatment and an absence of a saturation value after treatment. Such a difference reflects an increase in the parameters (less organization, more chaoticity), which are needed in order to describe the dynamics characterizing the disease free mucosa. In terms of the pathophysiology of gastric lesions the observed difference that appeared in the MGG of the lymphoma free mucosa can be expressed as an alteration of the high rhythmicity and synchronization, which characterize lesional versus malignancy free mucosa.

Our data imply that biomagnetic measurements and the nonlinear analysis are optimal procedures in assessing and differentiating MALT type gastric malignancies.

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The SQUID and the Role of the Pineal Gland for the Evaluation of Patients with CNS Disorders before and after External Magnetic Stimulation

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Abstract: Magnetoencephalogram (MEG) recordings of patients with CNS disorders were obtained using a whole-head 122-channel magnetometer SQUID and analyzed using Fourier statistical analysis. External transcranial magnetic stimulation in the order of pico Tesla (pTMS) was applied on the above patients with proper characteristics (magnetic field amplitude :1-7.5pT, frequency :the α-rhythm of the patient: 8-13 Hz) which were obtained with MEG recordings prior to pTMS. The MEG recordings after the application of pTMS shown a rapid attenuation of the high abnormal activity followed by an increase of the number of the low frequency components toward the patients α-rhythm. The possible mechanisms by which the magnetic stimulation is acting are discussed. Specifically, since the pineal gland has been shown to be a magnetosensitive organ and since it exerts an inhibitory action in animals and humans it will discussed its potential role in the brain on the effects of external magnetic stimulation.

Key- Words: - SQUID, MEG, Parkinson, Epilepsy, Multiple Sclerosis

1. Introduction

It is known that transmembrane, intracellular and extracellular neuronal currents each produce surrounding magnetic flux, however, the magnetic field recordable outside of the head is a selective reflection of the intracellular currents flowing in the apical dendrites of pyramidal cells parallel to the skull surface. The magnetic field generated by a single neuron is almost negligible, however, when several thousands of nearby cells are synchronously active, the summated produced extracranial magnetic field typically achieves a magnitude of only a few hundred femto-Tesla ($1fT=10^{-15}$) which compared with the strongest neuromagnetic signals, like those associated with epileptic spikes or any other abnormal neural activities, are only a few thousands femto-Tesla in magnitude [1-4]. These magnetic fields can be measured with sensors that take advantage of how the strength of a magnetic field changes as a function of the distance from its source. Such magnetic fields emitted from the brain are very weak (of the order of pT; $1pT=10^{-12}T$), so very sophisticated devices must be utilized in order to detect and record these fields .These devices are the ones which are based on the Josephson effect of superconductivity [5]. Such sophisticated device is the magnetometer SQUID the name of which comes from the initials of the following words (Superconductive Quantum Interference Device). The SQUID has the ability to detect magnetic fields of the order of 10^{-15}T which is much smaller than the magnetic field of the earth which is $5X10^{-5}$ T or 50μT. The signal measured by the SQUID is a time varying voltage waveform that reflects local changes in the magnetic flux as a function of time. This signal is called magnetoencephalogram (MEG) if it is measured the brain emitted magnetic fields and it is very similar to the electroencephalogram (EEG) which it is measured the brain emitted electric fields.

The MEG is presently regarded as the most efficient method for recording the brain activity in real time for many reasons. Compared with the EEG, the MEG has unique sensitivity to the CNS disorders and normal functions of the brain. In addition, the MEG offers functional mapping information and measurement of brain activity in real time, unlike CT and MRI and fMRI which only provide structural, anatomical and metabolic information. With the MEG the brain is seen in 'action' rather than viewed as a still image. Last,

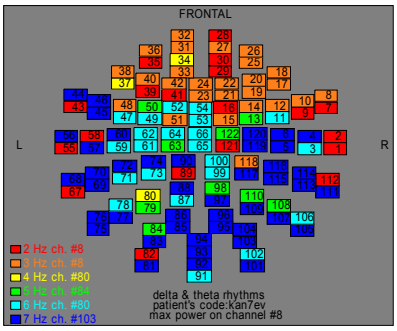
and most important is that the MEG has far more superior ability to resolve millisecond temporal activity associated with the processing of information which is the main task of the brain.
Thus, using the SQUID both normal spontaneous rhythms and pathological activities are readily identified in MEG waveforms as we do with the EEG waveforms. Whereas, MEG signals reflect current flow in the apical dentrites of pyramidal cells oriented tangential to the skull surface, EEG signals reflect both tangential and radial activities [6].
The goal of this paper is to report the above mentioned potential superiority of the MEG signals obtained in the diagnostic evaluation of CNS patients before and after the application of low intensity external transcranial magnetic stimulation using Fourier statistical analysis in frequency domain.
The transcranial magnetic stimulation (TMS) as currently used, was introduced by Barker et al. [7]. The TMS provided for the first time as a non-invasive, safe and painless method of activating the human motor cortex and assessing the integrity of central motor pathways. Since its introduction, the use of TMS in clinical neurophysiology, neurology, neuroscience and psychiatry has spread widely, mostly in research applications, but increasingly with clinical aims in mind [8,9]. On the other hand Anninos and his associates [3,4,10] applied also with a special electronic device [11] weak external TMS (in the order of pico Tesla) with proper field characteristics (intensity : 1-7.5 pT, frequency : 8-13 Hz) in the frontal, occipital and temporal lobes of the patients with CNS disorders. This electronic device consists of a low voltage generator, which can produce low frequencies, from 2-13 Hz, to a group of 32 coils of 1cm in diameter [11]. The 32 coils are enclosed between two parallel plastic plane surfaces in such a way that the axis of the coils is situated perpendicular to these surfaces.
.
With this new medical tool we ought to ask ourselves what it can offer that established methods do not for diagnostic, prognostic and therapeutic parts of clinical neurology. A new neurological tool might have several benefits: establishment of a dominant frequency of the power spectra of the MEG obtained from each channel after the application of Fast Fourier Transform for each epileptic patient. Then, it was constructed a two dimension map for the spatial distribution of the above mentioned primary dominant frequencies from all power spectra over the scalp. Different colors in the map represent different dominant differential diagnosis earlier or with greater certainty for a given clinical presentation than existing methods; better prediction of the likely course of the disease; further support for sustained and intensive interventions; help in identification of the most suitable treatment strategy; or improvement of clinical outcome as a therapy itself.
The main clinical application of TMS concerns testing of the functional integrity of the corticospinal tract in patients with disorders affecting the CNS. Use of standard TMS in these neurological disorders provides information on detection of subclinical upper motoneuron involvement, localization of anatomical site of lesions, longitudinal monitoring of motor abnormalities during course of diseases, and valuable aid to differential diagnosis. There are interesting results in CNS dysfunctions using TMS treatment. By changing the frequency of stimulation, it may be possible to modulate cortical excitability for therapeutic benefit. Thus, the ability of TMS to measure and modify cortical activity offers possibilities to apply this method to clinical neurology, neurorehabilitation and psychiatry [13].

2. **TMS in clinical Neurology**

The TMS has been tested to study different forms of epilepsies from generalized to focal epilepsies. The most common abnormality in all types of epilepsies that we have studied was an increased excitability with a reduction of intracortical inhibitory mechanisms. In order to test the effect of the application of TMS to all these types of epilepsies MEG measurements were performed using the whole-head 122-channel SQUID gradiometer device operated at low liquid helium temperatures (4K0). Recordings were taken in an electromagnetically shielded room in order to avoid extraneous electromagnetic noise. The MEG recordings were obtained with sampling frequency of 256Hz and filtered with cut-off frequencies between 0.3 to 40 Hz. The time taken for each recording was 2min in order to ensure alertness for each subject.
A software program was developed in our lab in order to detect the amplitude of the primary frequencies (red=2Hz, pink=3Hz, yellow=4Hz, green=5Hz, blue≥6Hz). Figures 1 and 2 respectively demonstrate the maps of the spatial distribution of the 1st dominant frequency over the scalp for a particular epileptic patient randomly selected from a large pool of epileptic patients and a normal volunteer before the application of external pTMS. As it is observed prominent low

frequencies can be seen in the map for the epileptic patient, whereas in the control volunteer map show that the frequency range was ≥6 Hz in the majority of channels indicating the appearance of a-rhythm which is the control rhythm for normal subjects. Thus, the spatial distribution of the amplitude of the dominant frequencies of all power spectra in the maps of all examined epileptic patients tend to be

Figure 1. This figure gives the spatial distribution of the first dominant power frequency amplitude for one epileptic patient in which it is seen prominent low frequencies in most of the brain areas.



located over a wide area in the low frequency domain, whereas in normal subjects the spatial distribution of the amplitude for the 1st dominant frequencies for all power spectra is clustered in the map showing domains with higher frequencies.

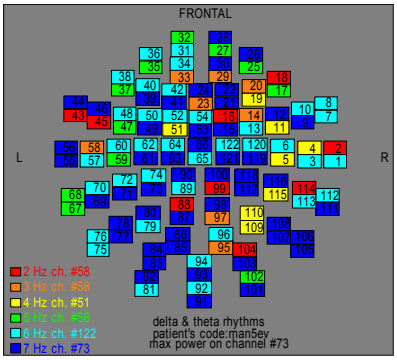


Figure 2. This figure shows the spatial distribution of the power spectra for the first dominant

frequency amplitude from a normal subject. In this map it is observed in all brain regions prominent frequencies ≥6Hz.

There are interesting results in the short-term treatment by daily sessions by applying TMS(magnetic intensity:1-7.5pT; frequency: the a-rhythm of the patient:8-13Hz) in all epileptic patients including the randomly selected one stated above. This was done by placing the coils of the device [11] on the patient's scalp for a total of 6 minutes (2 minutes over each of the following areas: left and right temporal regions, frontal and occipital regions, and over the vertex). The time between the first MEG and the MEG obtained after the application of the TMS was about one hour. By applying the same shoftware program, as it was stated above, we can detect the primary dominant

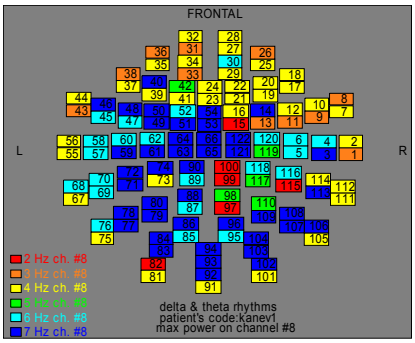


Figure 3. This figure shows the spatial distribution of the power spectra for the first dominant frequency amplitude from the MEG records after TMS for the epileptic patient of Fig.1.

Similar studies we have performed also with Parkinson's disease patients before and after the application of external transcranial magnetic stimulation. All Parkinson patients had diagnosed independently to suffer from idiopathic Parkinson disease (PD) and none of the patients had a history of other neurological disease other than PD. Biomagnetic MEG measurements were performed, as before using the whole-head biomagnetomer 122 channel SQUID in a magnetically shielding room of low magnetic noise.
During the MEG recordings the subjects, as before, were sitting in a chair with their heads covered by a

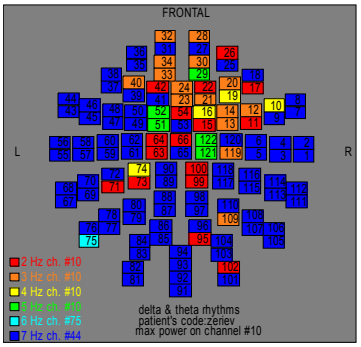


Figure 4. The spatial distribution of the power spectra for the first dominant amplitude frequency obtained from the MEG records of a Parkinson patient before TMS.

As it can be seen from Fig.4 the spatial distribution of the power spectra for the first dominant frequency is characterized by low frequencies. On the other hand the application of external magnetic stimulation on the Parkinson patient of Fig.4, as it helmet shaped dewar. Four indicators coils attached to the patient head determined the exact position of the head with respect to the MEG sensors.
The exact positions of the coils were determined using a three dimensional digitizer. In Figure 4 it is shown the spatial distribution of the power spectra for the first dominant frequency amplitude obtained from the MEG records from a particular PD patient selected randomly from the pool of all examined Parkinson patients prior to the application of external magnetic stimulation.

is seen in Figure 5 shows that the power spectra distribution of the first frequency amplitude is cluster in domains showing higher frequency as it should be for normal subjects

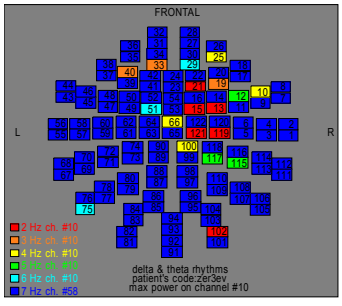


Figure 5. This figure is showing the distribution of the power spectra for the first dominant frequency amplitude of the MEG records obtained from the Parkinson patient of fig.4 after the application of TMS.

To confirm that the responses to TMS were reproducible, as it is shown in Fig.3 and fig.5, the patients were instructed to apply TMS with the same characteristics, with those used in our laboratory, nightly at home. Since this resulted in the same reaction to the one obtained in our laboratory and since this effect was sustained for a period more than a year, we preliminarily concluded that the application of the TMS is a non-invasive, safe and efficacious modality in managing patients with CNS disorders.

3. Results

The results reported in this section are representative for the group of epileptic and Parkinson patients that were diagnosed for the last five years using the whole-head 122 channel SQUID. The first case presented here refers to 30-years old patient suffering from idiopathic epilepsy since the age of 11. When he was first visited our Laboratory (in September 2001), he was manifesting five to 10 seizures per day with loss of consciousness and without falling down. The use of MEG recordings with the 122 channel SQUID diagnosed as having generalized epilepsy as it is seen in the map of Figure 1. This figure is showing the spatial distribution of the power spectra for the first dominant frequency amplitude of the MEG recordings obtained from the scalp of the patient prior to the external magnetic stimulation.
After the application of external magnetic stimulation to the above epileptic patient, using the electronic device [11] with the specific characteristics in the magnetic field intensity and frequency, as were stated in the introduction, we can obtain again a new MEG record.

Figure 3 illustrates the effect of the spatial distribution for the power spectra of the first dominant frequency amplitude for this randomly selected epileptic patient from the pool of epileptics patients examined in our laboratory. As it is seen the new map is characterized by a cluster of higher frequencies similar to the map of normal subjects. In addition, we have seen that this procedure was associated with the attenuation in the frequency and severity of patient seizures.
The second case is for a Parkinson patient selected also randomly from the group of Parkinson patients diagnosed in our laboratory. All these patients have diagnosed to suffer from idiopathic tremor, rigidity, and dyskinesia on the basis of clinical observations and routine EEG recordings. The use of MEG recordings again with the whole-head 122 channel

SQUID we obtained the map of Figure 4. This map is showing the spatial distribution of the power spectra for the first dominant frequency amplitude of the MEG recordings obtained from the Parkinson patient scalp prior to the external magnetic stimulation. This Parkinson patient was selected randomly from the whole group of Parkinson patients diagnosed with the 122 channel SQUID in our laboratory.

In this map we noticed that there are certain areas where the first dominant frequency amplitude, obtained from the power spectra of the MEG recordings from the scalp of the above mentioned Parkinson patient, are showing domains of low frequency . After the application of external magnetic stimulation to the above selected Parkinson patient we can record again a new MEG as before.

Figure 5 illustrates again the effect of the spatial distribution for the power spectra of the first dominant frequency amplitude which is characterized by similar cluster of higher frequencies similar to the map seen in normal subjects. Furthemore, it was noticed that with this procedure the Parkinson patients resulted in rapid attenuation of Parkinson symptoms.

4. Discussion

The brain is a complex dynamical system, so multichannel measurements are necessary to gain a detailed understanding of its behavior. Such multichannel measurements include optical brain images, multielectrode recordings, functional magnetic resonance imaging, MEG, etc[14-16].

In the MEG recordings, weak magnetic fields of the order of tens of fT/√Hz generated by electric currents in the brain are measured using the SQUID's detectors placed on the skull of the patients. The MEG is a noninvasive imaging technique, applicable to the human brain with temporal resolution approximately ~1ms [17]. Several authors have demonstrated the importance of the MEG in the investigation of normal and pathological brain conditions during the last decade [18-22]. The major advantage of MEG over EEG is that MEG has higher localization accuracy. This is due to the fact that different structures of the head (brain, cerebrospinal fluid, skull and scalp) influence the magnetic fields less than they influence the volume current flow that causes the EEG. Additionally, the MEG is reference free, so that the localization of the sources with a given precision is easier for the MEG than it is for EEG [23]. Frequency analysis is being increasingly applied in the investigation of CNS disorders before and after the application of low external magnetic fields with several advantages over the time domain technique [24]. Low frequency activities have been observed in our maps and occurred as thalamocortical synchronization transiently during wakefulness, under specific conditions of mental and emotional activity. Comparing all the maps which were obtained from the spatial distribution of the power MEG spectra for the first dominant frequency amplitude from all subjects, it can be seen that there is an increase number in the low first dominant frequency power amplitude for all subjects before the application of TMS, whereas the opposite is true for all subjects after the application of TMS.

These effects are due to the dopamine, which is produced and released by the dopaminergic neurons in the substantia nigra a region of the brain, which was found to reverse bradykinesia in animals and humans. Bradykinesia also includes difficulty initiating movement, sloweness in movement and paucity or incompleteness of movement, and which is markedly depleted in the case of PD[28]. There is evidence that melatonin which is the main hormone released from the pineal gland stimulates dopamine release from the substantia nigra which stimulates GABA(Gamma-Aminobutyric Acid) which is a major inhibitory neural transmitter in CNS and is closely related to epilepsy. When the activity of GABA is stimulated by dopamine action this inhibits the neural cells to overacting and to have seizures. However,when the activity of GABA is reduced we don't have enough inhibition and the neural cells start to overacting and as a result we have seizure activity. The above mentioned burst of the neurotransmitter dopamine, from the substantia nigra , goes to the striatum in the basal ganglia which are the motor parts of the brain which are involved in the pathogenesis of Parkinson disease and other neurological disorders of motor behavior. Thus, the melatonin stimulates catecholamines(dopamine and norepinephrine), serotonin and GABA and therefore reduction in its secretion my deplete cerebral concentration of these neural transmitters and thus facilitate the development of parkinsonism and other CNS dysfunctions.

Therefore, due to this beneficiary effect, the application of such external magnetic fields has been used recently by more and more scientists using transcranial and intracranial methodologies and have become convinced that it is proven to be a valuable tool for managing CNS disorders [25-27].

5. Conclusion

Although the beneficial effects of the application of TMS on the clinical picture in all CNS patients are well observed, the mechanisms underlying the efficacy of TMS remains an open question. One possible explanation of our findings is provided indirect support of our hypothesis that the beneficiary properties of the TMS are mediated via the pineal gland which is a magnetosensitive organ of our brain [3]. Taking this into account, the activity of this gland may be one of the crucial factors which determine and control the neural activity of all these patients suffering from CNS disorders. However, the question is difficult to be answered given the complexity of cellular, systemic and neuroendocrine effects of the TMS on biological systems and their potential impact on neurotransmitter functions. Despite all these facts, this method of magnetic stimulation may be considered as a very important noninvasive modality in the management of idiopathic CNS disorders.

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The Biological Effects of TMS in the Modulation of Seizures in Epileptic Patients

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Abstract: The goal of this study was to investigate the influence of transcranial magnetic stimulation (TMS) in epileptic patients using magnetoencephalographic (MEG) measurements and Fourier statistical analytic techniques. Our study population comprised with 10 men aged 19-56 years (mean: 39.7, SD: 10.5) and 10 women aged 15-53 years (mean: 34.7, SD: 12.6). For each patient the magnetic activity was recorded from a total of 64 points (32 points for each temporal lobe). TMS with proper field characteristics (intensity: 1-7.5 pT, frequency: the α-rhythm of the patient (8-13 Hz)) was applied in the frontal, occipital and temporal lobes for 2 to 6 minutes and the emitted MEG activity was recorded again. The application of TMS resulted in rapid attenuation of the high MEG activity and of the incidence of seizures in epileptic patients. The lower activity and the reduction of seizures after the application of TMS strongly support the beneficial effects of TMS in epileptic patients.

Keywords: MEG; SQUID; TMS; epilepsy; EEG

1.Introduction

The cerebral cortex is known to produce weak magnetic fields that can be recorded using the Superconducting Quantum Interference Device (SQUID). Such measurements are known as magnetoencephalogram (MEG). Unlike the electroencephalogram (EEG), the MEG is not subject to interferences from the tissues and fluids lying between the cortex and the scalp. Ionic movements throughout the neuronal cell body creating a current dipole follow changes in membrane potential. The orientation of the current dipole is a critical factor, which affects the measurement of magnetic fields. The MEG produced by such fields is exclusively created by a flow of electric currents tangential to the skull surface and therefore the signal will originate maximally from the cerebral sulci (where the pyramidal cells are more favorably oriented) and only minimally from the gyri surface where their orientation is less favorable [1-3]. As it was pointed out by Elger et al. [4] the single dipole model is not the most appropriate model for the conceptualization of seizure activity since: a) an epileptic focus generates different types of seizure activity; b) the brain area which generates an epileptic discharge varies and different neuronal populations may contribute to a single epileptic event; c) the synchronized potentials of "epileptic" neurons give rise to synchronized projected synaptic activity; d) the interictal activity may be localized only in a limited number of patients with seizure disorders. In order to avoid these difficulties we have proposed an alternative approach for the evaluation of the MEG recorded from patients with CNS disorders. Thus, instead of studying the surface distribution of the MEG in the time domain our method was based on investigating the surface distribution of the MEG in the frequency domain. This was proposed on the basis that the surface distribution of the spectral energy would exhibit patterns for specified locations of CNS disorders.

Transcranial magnetic stimulation (TMS) provides a non-invasive evaluation of distinct excitatory and inhibitory functions of cerebral cortex in humans [5]. Moreover, trains of regularly repeated magnetic stimuli can modulate the excitability of cortical networks [6]. For instance, 0.9–1 Hz repetitive TMS (rTMS) of the primary motor cortex (M1) produces a transient reduction in the excitability of

the neuron network generating the motor evoked potential (MEP) that outlasts the train itself [7]. Hence, it was argued that low-frequency (≤1 Hz) rTMS reduces cortical excitability. However, the effects of rTMS slower than 0.9–1 Hz on the same or similar indexes of M1 excitability are still unknown. At the same time, some studies suggest that rTMS at 0.3 or 0.5 Hz may have a therapeutic effect in epilepsy. First, [8] found that 0.3 Hz reduced the epileptic spike frequency in patients with mesiotemporal lobe epilepsy. Secondly, in two open studies, 0.33 Hz [9] and 0.5 Hz [10] rTMS have led to a decrease in seizure frequency in patients with intractable partial epilepsy. The mechanism hypothesized mainly consisted of a reduction in cortical excitability [9]. However, therapeutic effects of 0.3–0.5 Hz rTMS are yet to be replicated in randomized sham-controlled trials.

The aim of this study was to investigate the influence of TMS (in the order of pT) in epileptic patients using magnetoencephalographic (MEG) measurements and Fourier statistical analytic techniques.

2.Methods

The epileptic patients were referred to our Laboratory by practicing Neurologists. The examined group consisted of 10 men aged 19-56 years (mean: 39.7, SD: 10.5) and 10 women aged 15-53 years (mean: 34.7, SD: 12.6). All patients have been diagnosed independently to suffer from idiopathic epilepsy and had normal routine serum biochemical studies. Due to the limited resolution and low sensitivity of the EEG methods, a number of them appeared to have normal EEG, although the patients experienced seizures. The Hospital Ethics Committee approved the whole examination procedure and informed consent for the methodology of the study was obtained from all patients. Biomagnetic measurements were performed using a second order gradiometer SQUID (model 601 of the Biomagnetic Technologies Inc.), which was located in an electrically shielded room of low magnetic noise. The MEG recordings were performed after positioning the SQUID sensor 3 mm above the scalp of each patient using a reference system. This system is based on the International 10-20 Electrode Placement System and uses any one of the standard EEG recording positions as its origin (in this study we used the P3, P4, T3, T4, F3, and F4 recording positions). Around the origin (T3 or T4 for temporal lobes) a rectangular 32-point matrix was used (4 rows x 8 columns, equidistantly spaced in a 4.5 cm x 10.5 cm rectangle) for positioning of the SQUID [11-14]. The MEG was recorded from each temporal lobe at each of the 32 matrix points of the scalp for 32 sec and was digitized with a sampling frequency of 256 Hz. The MEG signal was band-pass filtered with cut-off frequencies of 0.1 and 60 Hz. TMS was applied in the frontal, occipital and temporal lobes using an electronic device [11-14] and the emitted magnetic activity were recorded again. The electronic device consists of a low voltage generator, which can produce low frequencies from 2-13 Hz to a group of coils of 1 cm diameter. The 32 coils are enclosed between two parallel plane surfaces in such a way that the axis of the coils is situated perpendicular to these surfaces. They are situated on the 32-point matrix, which is defined previously. The applied TMS carried similar field characteristics (intensity: 1-7.5 pT and frequency the α-rhythm of the patient (8-13 Hz)) with the ones emitted from the patient's brain prior of the application of TMS. The time between the 1st MEG and post-stimulation MEG is about an hour.

3.Results

None of the patients experienced side effects during or after the procedure. The above-discussed method for measuring the brain dysfunctions in epileptic patients before and after the use of TMS has been tested in over 300 patients [12,13]. In the present study we present only 20 patients randomly chosen giving their clinical response (Table 1).

4.Discussion

The after-effects of low-frequency rTMS provide some background to explain its reported therapeutic effects in some epileptic conditions. The latter are indeed characterized by an altered balance between excitatory and inhibitory influences at the cortical level [15-17]. Two open pilot studies suggested that 0.33 Hz [9] and 0.5 Hz [10] rTMS may reduce the seizure frequency in drug-resistant partial epilepsy. Moreover, 0.5 Hz rTMS prolonged the latency for development of pentylenetetrazol-induced seizures in rats [18]. By contrast, a small controlled trial found that 1 Hz rTMS produced a non-significant trend toward short-term seizure reduction [19]. Possibly, distinct effects of different rTMS frequencies could explain this difference.

The possible mechanisms by which magnetic fields have attenuated the patients' symptoms are still

controversial. However, one possible electrophysiological explanation for the efficacy of magnetic stimulation has been provided by the proposed "neural net model" [20], which suggests that magnetic stimulation causes a temporary neuronal inhibition in regions exhibiting paroxysmal discharges. The hypothesis is in accordance with data presented by other investigators. However, it is known that magnetic fields alter the activity of the pineal gland, which has been shown to regulate dopaminergic, and endogenous opioid functions. On a cellular level, the effects of magnetic fields on seizure activity maybe related to alterations in properties and stability of biological membranes and their transport characteristics including their intra- and extra cellular distributions and flux of calcium ions [21]. Another explanation for the management of epileptic activity using TMS is based on Morrell's hypothesis that every stimulus entering the brain is maintained for a certain period of time representing the short-term memory of the particular stimulus experience [22]. If the stimulus experience persisted for an extending period of time then the short-term memory of the presented stimulus is converted to the permanent memory of the stimulus. Based on this principle from neurophysiology it is possible to make the brains of epileptic patients to respond from their abnormal activities to normal ones using TMS of proper frequencies and intensities. In terms of the pathophysiology of epilepsy, the distortion of the high rhythmicity or abnormal synchronization and coherence of neural activity, which characterized brain activity of epileptic patients is an indication that we are modulating seizure activity in such a way that the characteristics of the time series are approaching the behavior of normal subjects.

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Table 1. The clinical data of the 20 patients and their response to TMS (N:normal, A:abnormal, P:partial normal)

SUBJECTS	AGE	AGE START	MEG DIAG BMF	MEG DIAG AMF	EEG DIAG BMF	EEG DIAG AMF	TYPE OF EPILEPSY	PLACEBO
WOMEN	19	5	A	P	P	N	GENERALIZED	NO EFFECT
	15	3	A	P	P	P	GRAND and PETIT MAL	NO EFFECT
	39	12	A	P	A	P	GENERALIZED	NO EFFECT
	49	28	P	N	N	N	GRAND MAL	NO EFFECT
	31	5	A	N	P	N	GRAND MAL	NO EFFECT
	30	4	A	P	A	P	GRAND MAL	NO EFFECT
	53	15	A	N	A	N	GRAND MAL	NO EFFECT
	48	2	A	N	A	N	GRAND MAL	NO EFFECT
	34	8	A	P	A	P	GRAND MAL	NO EFFECT
	29	15	A	N	P	N	GRAND MAL	NO EFFECT
MEN	40	15	A	N	A	P	GENERALIZED	NO EFFECT
	37	7	A	N	P	N	GENERALIZED	NO EFFECT
	34	15	A	N	A	P	GENERALIZED	NO EFFECT
	49	36	P	N	P	N	GRAND and PETIT MAL	NO EFFECT
	45	5	A	P	A	P	GRAND and PETIT MAL	NO EFFECT
	35	8	A	N	A	N	GRAND MAL	NO EFFECT
	33	14	P	N	P	N	GRAND MAL	NO EFFECT
	19	3	P	N	N	N	GRAND MAL	NO EFFECT
	56	10	P	N	P	N	GRAND MAL	NO EFFECT
	49	20	A	N	P	N	GRAND MAL	NO EFFECT

The Application of Non-Linear Analysis for Differentiating Biomagnetic Activity in Breast Lesions

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Abstract: The aim of this study was to investigate the biomagnetic activity obtained in benign and malignant breast lesions using non-linear analytic techniques. Magnetic recordings were obtained with a single channel biomagnetometer SQUID from 20 patients with palpable breast lumps: Of these 10 were invasive carcinomas and 10 were benign breast lesions. The exact nature of these lumps was determined histologically. The age of the patients with malignant tumors ranged from 42 to 64 compared with a range of 33-43 for patients with benign breast disease. Using the application of non linear analysis and dimensionality calculations we observed a clear saturation value for the dimension of malignant breast lesions and no saturation for the benign ones. The biomagnetic measurements with the SQUID and the application of non linear analysis, are promising procedures in assessing and differentiating breast tumors.

Keywords: SQUID; benign breast lesions; malignant breast lesions; non-linear analysis; biomagnetism

1.Introduction

Breast cancer is a major health problem. The worldwide incidence of the disease is increasing by 1.5% per annum. Depsite many detailed epidemiological studies, including a large number with biological measurements, the etiology of breast cancer remains unclear. Over 700.000 new breast cancers are diagnosed worldwide each year.

Breast cancer mortality rates have not changed during the past 60 years despite significant advances in screening mammography (1). It is tempting, therefore, to use novel technology in order to achieve a better understanding of breast oncology.

Screening programs involving periodic physical examination and mammography in asymptomatic and high-risk women increase the detection rate of breast cancer and may improve the survival rate. Unfortunately most women who develop breast cancer do not have identifiable risk factors and analysis of epidemiologic data has failed to identify women who are not at significant risk and would not benefit from screening. New less expensive screening techniques such as two-view mammography are being investigated in an attempt to reduce the cost of widespread screening.

The female breast, like any other living tissue, emits spontaneous magnetic activity caused by ionic movements across the plasma membrane (2). This activity, although exceedingly weak (it is about 10-8 of the earth's magnetic field which is equivalent to 50 μT), can be measured by means of a superconducting quantum interference device (SQUID) (2). The SQUID is a diagnostic tool capable of measuring the exceedingly weak magnetic fields emitted by the living tissues. The higher the concentration of living cells in the test area, the higher the biomagnetic fields produced and recorded from it. This technique has been used successfully for studying fetal heart (3,4), and more recently, in detecting ovarian tumors (5), brain activities and the hemodynamics of umbilical cord (6,7,8). The method is non-invasive because the SQUID is a receiver and not a transmitter.

Here we report the potential value of the biomagnetometer SQUID and the use of non linear analysis in assessing malignant and benign breast lesions.

2.Materials and Methods

Magnetic recordings were obtained from 20 patients with palpable breast lumps: Of these 10 were invasive carcinomas and 10 were benign breast lesions. The exact nature of these lumps was determined histologically (Table I). The age of the patients with malignant tumors ranged from 42 to 64 compared with a range of 33-43 for patients with benign breast disease. Only patients with

lesions in the right breast were included in this investigation. This was considered necessary so as to eliminated interference from the heart's magnetic activity which can affect the reliability of the measurements. Similarly, no patient in this series was subjected to fine needle aspiration cytology, as such an invasive procedure might increase biomagnetic activity. Women from both subgroups were comparable according body mass index (BMI). All lumps were located in the upper-outer quadrant of the breast and their diameter was less than 2 cm. All women were advised to stay still during the recording time. The temperature of all the examined women was within normal limits (approximately 37^o C). Informed consent for the study was obtained from all patients prior to the procedures. The study was approved by the hospital Ethics Committee.

The method used for the recording of magnetic activity has been described in detail elsewhere (9,10,11). In brief, we used a single channel SQUID second order gradiometer (DC SQUID model 601 of the Biomagnetic Technologies) which is produced in USA. The gradiometer operates at low liquid helium temperatures (4o K) on the basis of the Josephson effect of superconductivity (12), with a sensitivity of 95 pTesla/Volt at 1000 Hz. Recordings were taken in an electrically shielded room with the patient lying supine on a wooden bed, free of any metallic object so as to decrease the environmental noise and get better S/N ratio. In all patients five points were selected for examination. Point 5 (P5) was located at the very center of the breast lump, whereas points 1-4 (P1-P4) were located at the periphery of the examined area. For each point 32 recordings of 1-second duration each were taken with the SQUID detector placed 3mm above the recording position. This allows the maximum magnetic flux to pass through the coil with little deviation from the vertical direction. The duration of the above records is justified because the chosen time interval is enough to cancel out, on the average, all random events and to remain only the persistent ones. The sampling frequency was 256 Hz with a bandwidth of between 1 and 100 Hz. Using an AD converter, the analog signals were converted to digital ones and, after Fourier statistical analysis, the average spectral densities from the 32 records of magnetic field strength were obtained from each one of the five points measured in the frequency range 2-7 Hz. By convention, the maximum value was used when assessing the breast lesions. Operators were blinded to clinical and mammographic findings.

In order to investigate if there is any differentiation in the complexity underlying the dynamics that characterized the benign and malignant lesions, the dimensional analysis of the existing strange attractors was applied, using chaotic analysis approach.

Chaotic analysis of magnetic breast signals

Nonlinear analysis is a powerful technique for the estimation of the dimension of the strange attractor which is characterize the MMG (magneto-mammo-gram) time series obtained from patients with breast lesions.

For the estimation of the dimension of the strange attractor we used the method proposed by Grassberger and Procaccia (1983b), which is based on Takens (1981). Accordingly the dynamics of the system can be reconstructed from the observed MEG time series $B_i=B(t_i)$ (i=1,2...N). Then, the vector construction of V_i is given by the following equation:

$$V_i=\{B_i,B_{i+\tau},...,B_{i+(m-1)\tau}\} \qquad (1)$$

This equation gives a smooth embedding of the dynamics in a m-dimensional phase space. The evolution of the system in the phase space, once transients die out, settles on a submanifold which is a fractal set, the strange attractor. The existence of strange attractors related to the behavior of the system as chaotic or deterministic. The strange attractor can be described by a geometrical parameter, the correlation of fractal dimension D. This parameter is related to the number of variables required to define the attractor within the phase space and it can be estimated from an experimental time series by means of the correlation integrals C(r, m) defined as:

$$C(r,m)=\lim_{n\to\infty}(n(n-1)/2)^{-1}\sum_{\substack{i=1\\ i\neq j}}^{n-1}\sum_{j=1+i}^{n}\Theta(r-|V_i-V_j|) \qquad (2)$$

where $\Theta(u)$ is the Heaviside function defined as ($\Theta(u)=1$ for $u>0$ and $\Theta(u)=0$ for $u\leq 0$), m is the embedding dimension and n is the number of vectors constructed from a time series with N samples, given by the formula $n=N-(m-1)\tau$ (here τ is a delay parameter which is equal to the first zero crossing of the autocorrelation time of the MEG signal).

The correlation integral C(r, m) measures the spatial correlation of the points on the attractor and it is calculated for different values of r in the range from 0 to rmax . The rmax is equal to (m)1/2 (xmax-xmin), (assuming that xmax and xmin are the maximum and the minimum recorded values in the time series). For a chaotic system the correlation integrals should scale as C(r, m) ~ rD(m) . Thus, the correlation dimension D of the attracting submanifold in the reconstruction phase space is given by :

$$D = \lim_{\substack{r \to 0 \\ m \to \infty}} \partial(\ln C(r,m)) / \partial(\ln(r)) \quad (3)$$

In the case of a chaotic signal exhibiting a strange attractor, there is a saturation value, (plateau) in the graph of the slopes ∂ (lnC(r, m))/ ∂ (ln(r)) vs ln(r). This value remains constant, although the signal is embedded in successively higher-dimensioned phase spaces and gives an estimation of the correlation dimension of the attractor.

Using the above-described method the correlation dimension D of the selected MMG time series was estimated for the benign and malignant lesions. The purpose of this estimation was to investigate whether there is any biological differentiation in the dynamics in these two types of lesions.

3. Results

Table 1. Histological diagnosis of the 20 palpable breast lesions

	Type of lesions	
Benign (n=10)	Fibrocystic disease	5
	Duct ectasia	2
	Fibroadenoma	3
Malignant (n=10)	Invasive duct carcinoma of no special type (NST)	10

High amplitudes and rhythmicity characterize the MMG record from malignant breast lesion (Fig.1). The MMG obtained from benign breast lesion does not exhibit high amplitudes and rhythmicity of low frequency (Fig.2). The application of Grassberger-Procaccia algorithm to the MMG in malignant and benign breast lesions gives us the correlation integrals .We calculated the slopes ∂(Ln C(r,m))/ ∂(Ln(r)) v's Ln(r), for different values of the embedding dimension m, using dimensionality calculation on the MMG of the malignant and benign breast lesions. The mean value of 8.2±0.3 is the saturation point of the correlation dimension for the MMG of the malignant breast lesions, whereas in benign ones there is no clear saturation.

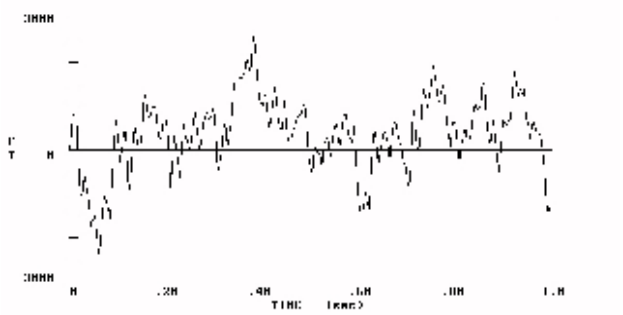

Fig.1. The MMG recorded from a malignant breast lesion for 1sec duration interval

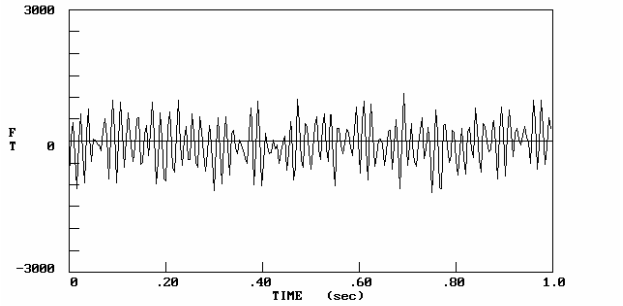

Fig.2. The MMG recorded from a benign breast lesion for 1sec duration interval

4.Discussion

The data, which we are shown in this study, although preliminary, present a novel approach of the MMG, which is a method of measuring the biomagnetic activity of breast diseases, and which with the use of non-linear analysis it is possible to differentiate benign and malignant breast lesions. The malignant tissues, by virtue of their rapid expansion and vascularity have increased ionic movements and therefore produce magnetic fields of higher intensity than slower growing benign breast tissues (2).

The differences reported in these studies are apparently due to malignancy itself and are not influenced to any extent by other factors such as the size of the tumor, the proportion of fat to glandular tissue, or the depth of the lesion within the mammary gland. The size of the lesion per se seems to have very little influence on the recordings obtained and, indeed, the largest lesion in the series was a benign fibroadenoma with a low

value. Equally, if there was to be any interference from the fat tissue surrounding the lesions, this should have an adverse effect on the magnitude of values obtained for the group of older patients, i.e. those with malignancies, since the proportion of fat to glandular tissue increases with advancing age. Undoubtedly, the closer the lesion to the skin surface the greater the values recorded, but there was no evidence that malignant lesions were lying more superficially than benign abnormalities. In addition, deep seated tumors are suitable for biomagnetic measurements as the probe used is sensitive to a depth of 4 cm. Artifacts that could affect results include patients' slight motions, but these, if any, would affect both groups of patients equally.

It has to be mentioned, however, that these results relate exclusively to palpable breast lumps and not to early inpalpable lesions. Furthermore, a much larger sample of patients is required before more firm conclusions can

be drawn. Despite these limitations, it appears that biomagnetic measurements with the use of non-linear analysis may prove a useful method in differentiating malignant and benign breast lesions.

Finally, the dimensionality calculations, which we have applied in MMG signals of malignant and benign breast lesions, were useful for the evaluation of the dynamics of these systems. Thus, by comparing the correlation dimension of the strange attractors underlying the dynamics of the malignant and benign breast lesions it is observed that there is a saturation value around 8 (Mean=8.2, STD=0.3) of the malignant and absence of a saturation value in benign lesions. Such a difference reflects an increase in the parameters which are needed in order to describe the dynamics which is characterized the benign breast lesion. In terms of the pathophysiology of breast lesions, the observed difference which appeared in the MMG of the benign breast lesions can be expressed as a distortion of the high rhythmicity, and synchronization which is characterized the malignant versus benign breast lesions.

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Correlation between Biomagnetic Measurements and Doppler Findings in the Differentiation of Uterine Myomas

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Abstract: To elucidate the hemodynamics of the uterine artery myomas by use of Doppler ultrasound and SQUID biomagnetometry. Twenty-nine women were included in the study. Eighteen of them were characterised with large myomas whereas 11 of them with small ones. Uterine artery waveform measurements were evaluated by use of Pulsatility Index (PI) (normal value PI<1.45). Biomagnetic signals of uterine arteries myomas were recorded and analyzed with Fourier analysis. The biomagnetic signals were distributed according to spectral amplitudes as high (140-300 ft/√Hz), low (50-110 ft/√Hz) and borderline (111-139 ft/√Hz). There was a statistically significant difference between large and small myomas concerning the waveform amplitudes (P<0.0005) and the PI index (P<0.0005). Specifically, we noticed high biomagnetic amplitudes in most large myomas (89%) and low biomagnetic amplitudes in most small ones (91%). It is suggested that the biomagnetic recordings of uterine artery myomas could be a valuable modality in the estimation of the circulation of blood cells justifying the findings of Doppler velocimetry examination.

Keywords: myomas; biomagnetic measurements; Doppler;SQUID;uterine artery

1.Introduction

Uterine myomas irrespective of whether they are small and asymptomatic (as in the postmenopausal women) or large and symptomatic (as in premenopausal women) considerably affect uterine artery blood flow velocity. Benign uterine leiomyomas are usually easily recognized with gray-scale ultrasonography, but may sometimes be difficult to differentiate from solid ovarian tumours. Doppler ultrasound is a diagnostic modality that has been used to characterize pelvic tumours, and transvaginal color and spectral Doppler examinations have been suggested to enable discrimination between benign and malignant adnexal masses [1-3]. Circulation of benign uterine leimyomas has been described by use of Doppler velocimetry. Some authors have tried to correlate the myoma volume and the blood flow circulation in the arteries of the wall of the myomas [4]. Much higher blood flow velocities were recorded in the arteries of large myomas than in small myomas [5]. PI values<1 in a pelvic mass have been taken to indicate malignancy. However Kurjak et al [6] reported the arteries of many myomas to manifest low blood flow impedance and high velocity blood flow. PI values<1 are common in uterine myomas and do not indicate malignancy. Uterine artery blood flow velocity reflects uterine perfusion, and low uterine artery PI values might originate from the need for increased blood supply in uteri with large myomas as a consequence of the increased uterine volume. In recent years SQUID biomagnetometry has proven to be most helpful in the study of hemodynamics of certain vessels by measuring the exceedingly weak magnetic fields emitted by circulating blood cells. The higher the concentration of blood cells in the tested area, the higher the biomagnetic fields produced and the higher the recorded measurements. This technique has been successfully used for studying the hemodynamics of the umbilical and uterine artery [7-9]. Our study aims to report the characteristics of the biomagnetic recordings obtained from uteri with myomas and to correlate them with the corresponding Doppler values in order to test the validity of the biomagnetometer SQUID in the evaluation of the hemodynamics of the blood flow circulation of uterine myomas.

2.Materials and Methods

The group study comprised 29 premenopausal women who were planed to undergo laparotomy because of symptomatic myomas. Eighteen of them were characterised with large myomas whereas 11 of them with small ones. The diagnosis of myomas was made by use of bimanual gynecologic examination as well as with both transabdominal and transvaginal gray-scale sonography. The transabdominal examination was necessary to adequately measure uterine size in women with large uterus. Transvaginal examination was performed with a woman in the lithotomy position. Myoma volume was expressed in cm3 and was calculated according to the formula length (cm) x depth (cm) c width (cm) x 0.5. A myoma was considered large if at least one of its diameters was > 5 cm; otherwise it was characterized a small one. If more than one myoma was found in the pelvis, the largest myoma was examined. The uterine arteries and myoma vascularization were visualized by the color Doppler technique. Blood flow velocity waveforms from both uterine arteries were obtained by placing the Doppler gate over the colour areas and activating the pulsed Doppler function. The main stem of the uterine arteries was examined lateral to cervix at the level of the internal os. The mean value from the PI obtained from the right and left uterine artery of each patient was recorded and correlated with the myoma volume and with the corresponding biomagnetic measurements. PI values were automatically calculated through the computer software after indicating the systolic and diastolic phase of the waveform. All women underwent hysterectomy or excision of the myoma and histologic diagnosis of a benign uterine myoma was made for all of them.

Biomagnetic recordings were obtained by a single channel second order gradiometer DC-SQUID (MODEL 601;Biomagnetic Technologies Inc., San Diego, USA) [7-11]. To attenuate the influence of electromagnetic artifacts, the measurements were performed in a shielded room of low magnetic noise. The noise level in such environment was of the order of 50 fT/√Hz. During the recording procedure the patient was relaxed lying on a wooden bed and the recordings were performed after positioning the SQUID sensor 3 mm above the target area. Five points were selected for examination according to the myoma topography made by use of gynecologic and ultrasound examination. Point 5 was located at the very center of the myoma, whereas points 1-4 were located at the periphery of the target area. For each point 32 recordings of 1-second duration each were taken and digitized by a 12 bit precision analogue-to – digital converter with a sampling frequency of 256 Hz. The biomagnetic signals were band-pass filtered, with cut-off-frequencies of 0.1-100 Hz. The associated Nyquist frequency limit, with the above-mentioned sampling frequency, is therefore 128 Hz, which is well above the constituent frequency components of interest in biomagnetic recordings and avoids aliasing artifacts. Informed consent for the study was obtained from all the patients prior to the procedure. The biomagnetic signals were distributed according to spectral amplitudes as high (140-300 ft/√Hz), low (50-110 ft/√Hz) and borderline (111-139 ft/√Hz). Statistical analysis was obtained using t-test.

3. Results

The results for the biomagnetic data are indicated in Figure 1. The raw data were of high amplitudes in most (89 %) of the large uterine myomas and low amplitudes in most (91 %) of the small ones. In all cases the frequencies considered were distributed in the

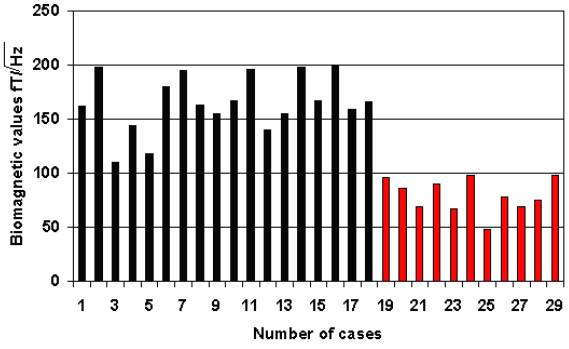

Figure 1. The spontaneous magnetic activity generated from the 29 women with myomas

range 2-7 Hz. The corresponding spectral densities of the magnetic field were shown after statistical Fourier analysis: these were of high spectral amplitudes in the apparently large myomas and of low spectral amplitudes in small ones. The maximum total average of spectral amplitudes emitted by the large myomas was: 165.11±26.54 fT/ √Hz whereas in the small ones 79.45±15.81 fT/ √Hz .The above difference was of statistical significance ($P<0.0005$, t-test). Table 1 presents the mean values of the myoma volume, the uterine artery PI and the biomagnetic amplitudes in the two study groups. A statistically significant difference

was observed in the PI values obtained from large and small myomas respectively (P<0.0005, t-test). Higher PI values were recorded in the uterine arteries of uteri with small myomas, while lower PI values were observed in the uterine arteries supplying large myomas.

Table 1. Mean values of myoma volume, uterine artery PI and biomagnetic amplitude in the two study groups.

	Volume	PI	Biomagnetic amplitude (fT/ √Hz)
Large myoma	287.21 (99.8-1119.4)	43.12 (29.6-56.25)	165.11±26.54
Small myoma	1.07 (0.67-1.29)	2.01 (1.19-2.76)	79.45±15.81
P(t-test)	<0.0005	<0.0005	<0.0005

4.Discussion

The data presented in this study, although preliminary, suggest a potential validity of biomagnetism in the differentiation of uterine myomas. This is not unexpected as malignant tissues, by virtue of their rapid expansion, vascularity and thus increased ionic movements produce magnetic fields of higher intensity than normal tissues [12].

It is well known that tumor hyperemia is related to new blood vessel growth (neovascularization) as well as to dilatation of previously existing vessels. Viable tumor cells release diffusible angiogenic factors, which stimulate new capillary growth and endothelial mitosis in vivo [13] even when tumor cell proliferation has been arrested by irradiation [14]. Folkman et al [15] proposed a hypothesis that "once tumor take occurs", every further increase in tumor cell population must be preceded by an increase in new capillaries which converge upon the tumor in early growth. According to this concept, a small focus of tumor cells could not increase indefinitely without the induction of angiogenesis. Furthermore, there is strong evidence that growth of solid tumors beyond a few millimeters in diameter depends on the induction of functional microcirculation from the surrounding host tissue. It is obvious that malignant tumor induces growth of the independent and characteristic vascular network on its own. The tumor vasculature is highly heterogeneous and does not conform to standard normal vascular organization (i.e. artery, to arteriole, to capillaries, to postcapillary venule, to venule, to vein). A key difference between normal and tumor vessels is that the latter are dilated, saccular and tortuous, and may contain tumor cells within the endothelial lining of the vessel wall [16].

It has been well established in previous studies that low uterine artery PI values are present in uteri with myomas. This may be an effect of increased uterine size and not necessarily an effect of the myomas per se. Differences in the uterine artery PI values might also reflect differences in the women's menstrual status (pre-menopausal vs menopausal women). In this study all women were pre-menopausal and the difference in PI values between the two groups is more likely to be explained by the difference in the myoma size.

Doppler velocimetry studies on myoma vessels (both capsule and core vessels) have shown that PI values < 1.0 are common in uterine myomas and do not indicate malignancy. This eliminates the role of Doppler study in the discrimination of benign uterine myomas from other malignant pelvic tumors. Biomagnetic recordings obtained from benign and malignant tumors of various organs (eg breast, ovary) proved to be helpful in the differentiation of the tumor's biologic behavior [10, 11]. Further biomagnetic studies on uterine myomas might elucidate the value of biomagnetism in the differential diagnosis of benign and malignant pelvic tumors. The data presented in the study, although preliminary, justify a novel approach to biomagnetism and suggest that this imaging modality of measuring the magnetic activity of uterine artery circulation can be potentially exploited in follow up of women with myomas. The method is a non-invasive procedure, well tolerated by the women, rapid and easy to interpret. Nevertheless, further studies in large numbers of women are needed in order to evaluate the role of biomagnetometry in the differentiation of uterine myomas and to investigate its potential role as an adjunct procedure to established diagnostic methods.

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Biomagnetic Findings in Gynaecologic Oncology (our experience)

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Abstract: This study aimed to investigate biomagnetic activity in benign and malignant ovarian and breast diseases using a biomagnetometer SQUID. Magnetic recordings were obtained from 30 patients with palpable ovarian lesions (14 of them were invasive carcinomas, and 16 were benign ovarian lesions) and 21 patients with palpable breast lumps (17 of them were invasive carcinomas and 4 were benign breast lesions). We used a one channel biomagnetometer SQUID (Superconducting QUantum Interference Device), in order to measure the magnetic field from benign and malignant ovarian and breast diseases. Interestingly, the ovarian and breast lesion waveforms and the corresponding spectral densities were of high amplitude in most malignant lesions, and of low amplitude in most benign diseases. These findings were of statistical significance ($p < 0.001$). It is suggested that biomagnetic measurements of benign and malignant ovarian and breast diseases, which is an entirely new application of SQUID technology, is a promising procedure for assessing tumors.

Keywords: SQUID; benign breast lesions; malignant breast lesions; benign ovarian lesions; malignant ovarian lesions

1.Introduction

Ovarian malignant neoplasms are the most common gynecological pelvic malignancy in most Western countries. The advent of vaginal ultrasound screening methods for ovarian cancer has made the ovaries more accessible. Dramatic changes in ovarian tissue vascularity during oncogenesis are mediated by numerous angiogenic factors and can be detected by using flow data from color Doppler. Malignant tumor vessels are usually dilated, saccular, and tortuous, and may contain tumor cells within the endothelial lining of the vessel wall. In view of the fact that ovarian malignancy is still an insidious and intractable disease, which is usually diagnosed at a late stage, is correlated with high rate of mortality and it is considered to be a "silent killer" and since there is a rather high incidence of these malignant tumors in the menopause, all means should be used to detect this disease at an early stage. Ovary lesions, like any other living tissue, emit spontaneous magnetic activity caused by ionic movements across the plasma membrane.

Breast cancer mortality rates have not changed during the past 60 years despite significant advances in screening mammography (1). Screening programs involving periodic physical examination and mammography in asymptomatic and high-risk women increase the detection rate of breast cancer and may improve the survival rate. Unfortunately most women who develop breast cancer do not have identifiable risk factors and analysis of epidemiologic data has failed to identify women who are not at significant risk and would not benefit from screening. New less expensive screening techniques such as two-view mammography are being investigated in an attempt to reduce the cost of widespread screening.

It is tempting, therefore, to use novel technology in order to achieve a better understanding of breast oncology. The female breast, like any other living tissue, emits spontaneous magnetic activity caused by ionic movements across the plasma membrane (2). The SQUID is a diagnostic tool capable of measuring the exceedingly weak magnetic fields emitted by the living tissues. The higher the concentration of living cells in the test area, the higher the biomagnetic fields produced and recorded from it. This non-invasive technique has been used successfully for studying fetal heart (3,4), brain activities and the hemodynamics of uterine artery and umbilical cord (5,6,7).

2. Methods

Biomagnetic recordings were obtained by a single channel second order gradiometer DC-SQUID (MODEL 601;Biomagnetic Technologies Inc., San Diego, USA). To attenuate the influence of electromagnetic artifacts, the measurements were performed in a shielded room of low magnetic noise. During the recording procedure the patient was relaxed lying on a wooden bed and the recordings were performed after positioning the

SQUID sensor 3 mm above the target area. Thirty patients with palpable ovarian lesions were examined. Of these14 were invasive carcinomas, and 16 were benign ovarian lesions .For the ovarian examination, 5 points were selected for examination. Point 5 was located at the very center of the ovarian lesion, whereas points 1-4 were located at the periphery of the target area. Twenty-one patients with palpable breast lumps were included in the study. Of these 17 were invasive carcinomas and 4 were benign breast lesions. Only patients with lesions in the right breast were included. This was considered necessary so as to eliminated interference from the heart's magnetic activity, which can affect the reliability of the measurements. For each point 32 recordings of 1-second duration each were taken and digitized by a 12 bit precision analogue-to –digital converter with a sampling frequency of 256 Hz. The biomagnetic signals were band-pass filtered, with cut-off-frequencies of 0.1-100 Hz. The associated Nyquist frequency limit, with the above-mentioned sampling frequency, is therefore 128 Hz, which is well above the constituent frequency components of interest in biomagnetic recordings and avoids aliasing artifacts. Informed consent for the study was obtained from all the patients prior to the procedure.

3. Results

The ovarian lesions raw data were of high amplitudes in most (95%) of the malignant ovarian lesions and low amplitudes in most (94%) of the benign ovarian diseases (Figures 1,2). In all cases the frequencies considered were distributed in the range 2-4 Hz. The corresponding spectral densities of the magnetic field were shown after statistical Fourier analysis: these were of high spectral amplitudes in the apparently malignant ovarian neoplasms and of low spectral amplitudes in benign ones. The above findings were of statistical significance ($p<0.001$).

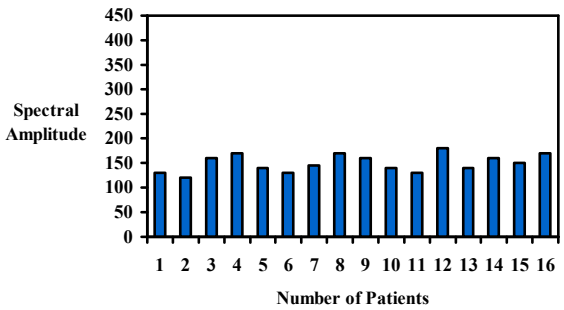

Figure 1.The spontaneous magnetic activity generated from the 16 palpable benign ovarian diseases

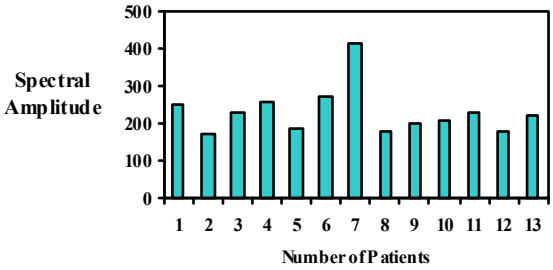

Figure 2. The spontaneous magnetic activity generated from the 14 malignant ovarian diseases

Benign lesions	
Fibrocystic disease	2
Fibroadenoma	1
Duct ectasia	1
Malignant disease	
Invasive duct carcinoma of no special type	14
Medullary carcinoma	1
Mucinous carcinoma	1
Invasive lobular carcinoma	1

Table 1. Histological diagnosis of the 21 palpable breast lesions

The waveforms of the magnetic field emitted from malignant breast disease had high amplitudes with spectral amplitudes in the order of 1800 fT/√Hz, whereas from benign breast disease had low amplitudes with spectral amplitudes in the order of 250 fT/√Hz. These findings were of statistical significance ($p<0.001$).

4. Discussion

It is well known that tumor hyperemia is related to new blood vessel growth (neovascularization) as well as to dilatation of previously existing vessels. Viable tumor cells release diffusible angiogenic factors, which stimulate new capillary growth and endothelial mitosis in vivo even when tumor cell proliferation has been arrested by irradiation (8). Folkman (9) proposed a hypothesis that "once tumor take occurs", every further increase in tumor

cell population must be preceded by an increase in new capillaries which converge upon the tumor in early growth. According to this concept, a small focus of tumor cells could not increase indefinitely without the induction of angiogenesis. Furthermore, there is strong evidence that growth of solid tumors beyond a few millimeters in diameter depends on the induction of functional microcirculation from the surrounding host tissue. It is obvious that malignant tumor induces growth of the independent and characteristic vascular network on its own. The tumor vasculature is highly heterogeneous and does not conform to standard normal vascular organization (i.e. artery, to arteriole, to capillaries, to postcapillary venule, to venule, to vein). A key difference between normal and tumor vessels is that the latter are dilated, saccular and tortuous, and may contain tumor cells within the endothelial lining of the vessel wall (10).
It seems, therefore that biomagnetic measurement of the ovarian and breast lesions may prove a useful method in detecting carcinomas.

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Biomagnetic Findings in Perinatal Medicine (our experience)

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Abstract: This study provides an overview of our experience in the application of biomagnetism in perinatal medicine. We provide a brief description of our research work in fetal magnetoencephalography, fetal arrhythmia, hemodynamics of the umbilical and uterine arteries, providing a new approach of biomagnetism as a non invasive imaging modality in the investigation of perinatal complications.

Keywords: SQUID, fetal MEG, fetal arrhythmia, hemodynamics of umbilical cord, hemodynamics of uterine arteries

1.Introduction

It is commonly known that the vascular system of mother and placenta plays an important role in the intrauterine development of the fetus. The umbilical cord arteries of newborns delivered by mothers with preeclampsia contain more than twice the amount of collagen and markedly less elastin in comparison to the corresponding arteries of newborns delivered by healthy mothers. Pathologic uteroplacental vasculature causes decreased uteroplacental perfusion and may explain the reduced placental weights and the intrauterine growth retardation seen in most – but not all- cases of preeclampsia. Preeclampsia and IUGR are major causes of maternal and neonatal morbidity and mortality. Preeclampsia complicates 5%-7% of pregnancies in USA, while IUGR affects 3%-7% of all pregnancies worldwide and is the second common cause of perinatal mortality –after preterm labor- in the developed countries [1].

The wide application of fetal heart monitoring in the obstetric practice has led to the increased recognition of fetal heart dysrrhythmias. A variety of techniques have been employed for the assessment of heart rate disorders, but only the electrocardiogram (ECG) can properly characterize the abnormality. However, though ECG is the mainstay of cardiology, its usefulness in cardiology is of limited value. This is due primarily to the poor signal quality of fetal ECG's recorded from the maternal surface, which typically show low amplitude and strong maternal interference [2]. M-mode echochardiography has been implemented in the diagnosis of fetal arrhythmias, but this method requires considerable expertise and good tracings are often difficult to obtain because of fetal movements. The duration of examination is often prolonged especially when the fetus is in an unfavorable position. Tracings obtained during early pregnancy are often unsatisfactory because of the small size of the fetal heart [3]. Furthermore, differential diagnosis of an arrhythmic event may be difficult because analysis of the signal morphology is not possible. Pulsed Doppler velocimetry of the fetal abdominal aorta and inferior vena cava provides with accurate diagnosis of the different types of congenital fetal arrhythmias. However, interpretation of the waveforms requires significant acquisition and the method has indication only in high-risk pregnancies due to the destructive cumulative biologic effect on fetal tissues [4].

Up to now it has not been possible to assess fetal brain function directly while the membranes are intact. Several indirect methods are in clinical use such as cardiotocography, biophysical profile, amniotic fluid examination, Doppler sonography, hormone analysis, and ultrasound investigations of fetal growth and fetal movements. Direct measurements of the brain's magnetic activity have important advantages over electroencephalographic (EEG) recordings [5]. The magnetoencephalographic (MEG) measurements provide higher temporal and spatial resolution than EEG because magnetic fields are not distorted by flowing through the tissues [6]. As a consequence, significant MEG activity can often be recorded in the absence of conventional EEG abnormalities [7].

In recent years SQUID biomagnetometry has proven to be most helpful in the study of hemodynamics of certain vessels by measuring the

exceedingly weak magnetic fields emitted by circulating blood cells. The higher the concentration of blood cells in the tested area, the higher the biomagnetic fields produced and the higher the recorded measurements. This technique has been successfully used for studying hemodynamics of the uterine and umbilical artery, fetal heart and brain activity [8-11].

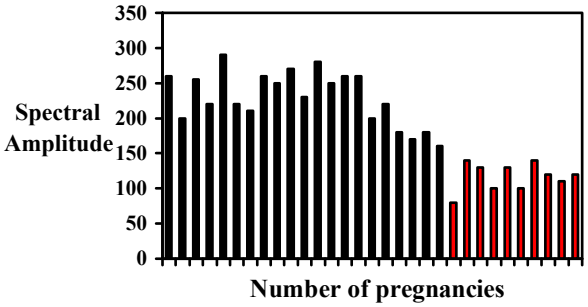
2.Methods

Biomagnetic signals were recorded using a single channel SQUID with a sensitivity of 95 pTesla/Volt at 1000 Hz (DC SQUID model 601, Biomagnetic Technologies, San Diego, USA). In order to minimize the incidence of stray electromagnetic radiation recordings were taken in an electrically shielded room of low magnetic noise. Patients were examined while lying in a lateral decubitus (either left or right) placement to avoid supine hypotension. Ultrasound scanner Doppler examination assessed prior to the procedure the exact placement of the target area in order to be sure that the biomagnetic signals from nearby vessels were excluded.
The SQUID probe was placed 3 mm over the exact position of the target area assessed by the Doppler examination in order to allow the maximum magnetic flux to pass through the coil with little deviation from the vertical direction. Thirty-two consecutive measurements, of 1 sec duration each, were taken. The sampling frequency was 256 Hz with a bandwidth ranging from 1 to 128 Hz. Conversion of the analog signals into digital recordings was accomplished by means of an AD converter on line with a computer. The average spectral densities from the 32 signals of magnetic field intensity were obtained using Fourier statistical analysis. The obstetricians were ignorant of the biomagnetic values [8-11].

The biomagnetometry of the umbilical artery

This study included 31 nonconsecutive pregnant women aged 17 to 38 years at full term gestation. Of these, 21 were apparently normal and 10 had pre-eclampsia. The waveforms of the magnetic field generated from the umbilical artery of a normal pregnancies had high amplitudes whereas from pre-eclamptic pregnancies had low.The difference was of statistical significance ($p<0.001$).

Figure 1: The distribution of the maximum magnetic field spectral amplitudes generated from the umbilical cord of 21 normal and 10 pre-eclampic pregnant women.



The biomagnetometry of the uterine artery

This study comprised with 60 nonconsecutive pregnant women aged 18-37 years. Of these 38 were apparently normal (gestational age 37-41 weeks) and 22 had pre-eclampsia (gestational age 37-38 weeks). The waveforms of the magnetic field generated from the uterine artery of the normal pregnancies had high values whereas from pre-eclamptic pregnancies had low amplitudes. The difference was of statistical significance ($p<0.001$). The rate of vaginal deliveries was higher in high biomagnetic amplitudes (83.7%) in compare with low ones (54.5 %). The results were statistically significant ($p<0.02$, chi-square). The rate of vacuum extraction was higher in low biomagnetic values (27.3%) in association with high ones (8.15%). There was a statistical significance difference in the percentages ($p<0.01$). There was a correlation of low biomagnetic values with a higher rate of operative deliveries (18.2%) in compare to the rate of high amplitudes (8.15%). The results of the statistical analysis of the percentages were not significant ($p<0.1$, chi-square)

Fetal arrhythmia

This study included 70 women, 19-41 years old with single normal uncomplicated pregnancies and gestational ages 25-32 weeks and 60 women, 23-43 years old with gestational age 26-35 weeks, who were treated with ritodrine for the risk of pre-term labor. M-mode echocardiography was also performed prior to magnetocardiography on all fetuses for the establishment of fetal arrhythmias. M-mode echocardiographic recordings of the cardiac motion with time where obtained using a single M-mode sampling line that intercepted both the atrial and the ventricular walls or the atria ventricular junction. M-mode echocardiography

revealed two cases of arrhythmias (one tachycardia and one bradycardia) in the corresponding sub-group. All these fetuses had a favorable outcome. The one with the ventricular extrasystoles (at least one extrasystole every 10 beats) was delivered at the 38th week of gestation with normal Apgar score and umbilical cord pH values. Tachycardia in the fetuses between 24-28 weeks disappeared after the 34th week of gestation. Tachycardia detected in one fetus at the 31st week of gestation disappeared within a few days after delivery, without further complications. M-mode echocardiography confirmed the diagnosis of bradycardia in one fetus (28th week of gestation). It was closely followed up, without signs of fetal distress or other complications. Bradycardia sustained after delivery and the neonate was referred to the cardiologists for further evaluation.

FMCG	Number of cases
Tachycardia	3
Bradycardia	1
S/V extrasystoles	5
Normal	121

Fetal magnetoencephalography

Two groups of pregnant women were examined aged 18-37 years at 37-40 weeks pregnancy. The first group consisted of 15 pregnant women with normal pregnancies and the second group comprised with 10 pregnant women with pregnancies complicated with pre-eclampsia. The fetal MEG measurements in pre-eclampsia had high values whereas in normal pregnancies were low. The difference was of statistical significance ($p<0.001$).

Table 1.Gravidae with normal pregnancies

Gestational age (weeks)	37	38	39	40
MEG (fT/√Hz)	323 336 347 351	383 385 374 365	400 403 404 406	410 409 423

Table 2. Gravidae with pregnancies complicated with pre-eclampsia

Gestational age (weeks)	Pre-eclampsia	MEG (fT/ √Hz)
37	Mild	379
	Severe	646
	Mild	344
	Mild	394
	Severe	622
38	Severe	822
	Severe	610
	Mild	438
39	Severe	696
	Mild	595

3.Discussion

It has been apparent from a number of studies that Doppler ultrasound has contributed greatly in the detection and management of high-risk pregnancies – such as the ones complicated by preeclampsia or IUGR – reducing perinatal morbidity and mortality by approximately 38% [12]. However, despite Doppler sonography's wide application in clinical practice in order to assess fetuses at risk for antepartum compromise, the sensitivity of the method differentiates greatly in different studies [13]. Maulik et al [12] suggest that Doppler sonography cannot act as a screening method to predict fetal distress and poor perinatal outcome. Golzarian et al [14] conducted an experimental study on biomagnetic recordings obtained before and after artificially induced intestinal ischemia. The study showed a strong correlation between reduced arterial blood flow and low biomagnetic amplitudes.

Fetal brain activity can be detected with MEG in normal and pre-eclamptic pre-term pregnant women [8-11] and in early and late gestational age [5,6]. The method provides certain advantages compared with conventional EEG due to its ability to record brain activity without direct contact with the head and the transparency of magnetic signals in passing through extracerebral fetal layers and the mother's abdomen. Therefore, it may provide a clinical tool for screening purposes in the antenatal surveillance of the fetal nervous system and especially in IUGR pregnancies for the prediction of perinatal outcome.

A number of studies reported in the past refer to the advantages which magnetocardiography presents compared to other diagnostic techniques such as M-mode echocardiography, two dimensional imaging, pulsed Doppler and color flow Doppler [15-17]. All the above mentioned studies confirm the diagnostic accuracy of magnetocardiography, especially

regarding functional heart disorders like cardiac arrhythmias, but, to our knowledge, there is only a limited number of reports in the literature evaluating the screening properties of the method. In conclusion, our data suggest a potential usefulness of SQUID biomagnetometry as a secondary diagnostic test in high-risk pregnancies or as an adjuvant to Doppler ultrasound. In the presence of abnormal biomagnetic activity, an intensified fetal surveillance should be considered mandatory on the basis of the likelihood of developing complications and early intervention might be required.

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Objective Evaluation of Taste with 122-Channel Biomagnetometer SQUID

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Abstract: The aim of this study was to evaluate the taste and the way that it is connected with the central nervous system (CNS) using a 122-channel SQUID biomagnetometer. Case report: A 27-years-old male healthy volunteer was referred to our department and his magnetoencephalography (MEG) was evaluated in three different states: physiological condition; with a sweet taste; with a salt taste. There was a differentiation in the spatial distribution of the frequencies over his head in all the states after the application of Fast Fourier Transform in his MEG data. This report provides novel insights into the evaluation of taste with the MEG systems and encourages more studies to be conducted.

Keywords: MEG, 122-channel, taste, Frequency domain, Fourier transform

1.Introduction

Time varying electric currents, in wires or brain cells, all produce time-varying magnetic fields. Even though transmembrane, intracellular and extracellular neuronal currents each produce surrounding magnetic flux, the neuromagnetic field recordable outside of the head is a selective reflection of intracellular currents flowing in the apical dendrites of pyramidal cells oriented parallel to the skull surface. This is because of the biophysical properties of neuronal currents and the volume conduction properties of the head.

The magnetic field generated by a single neuron is almost negligible; thus, when several thousands of nearby cells are synchronously active, the summated extracranial magnetic field typically achieves a magnitude of only a few hundred femto-Tesla. Even the strongest neuromagnetic signals-those associated with epileptic spikes-are only a few thousand femto-Tesla in magnitude [1-3].

The key to successful isolation of neuromagnetic signals is to use sensors that take advantage of how the strength of a magnetic field changes as a function of distance from its source. The output of each channel of the biomagnetometer is a time – varying voltage waveform that reflects local changes in magnetic flux as a function of time. The appearance of the MEG signal is very similar to that of the electroencephalogram (EEG) and both normal spontaneous rhythms and pathologic activities are readily identified in MEG waveforms. Whereas MEG signals reflect current flow in the apical dendrites of pyramidal cells oriented tangential to the skull surface, EEG reflects both tangential and radial activity [4-5]. A key to locating the neuronal population that generates a neuromagnetic signal of interest is adequate sampling of the spatial pattern of the magnetic field. This is most efficiently accomplished using a multichannel biomagnetometer system [6-8].

Physiology of taste

As it is known the basic taste sensations are sweet, salty, sour and bitter, though some researchers identify a fifth sensation, which is named after the Japanese word umami. The sensory organ of taste are the taste buds which can be identified in foliate papillae, vallate papillae and fungiform papillae. Taste buds can be also found on the tonsil, the esophageal orifice, hard palate, epiglotis, the anterior faucial pillar and the buccal mucosa. Saliva is also very important for taste because it allows the dilution of the chemical substances and the fine gustatory cells are bathed in it.

Taste is a very common sensation, which takes place when chemical substances cause depolarization of the taste receptor cells, which bare located in the taste buds in the oral cavity. Gustatory information is transferred to the central nervous system through the facial nerve (chorda tympani), the vagus nerve and the glossopharyngeal nerve. In specific, the anterior half of the tongue is innervated by the chorda tympani, the posterior third of the tongue is innervated by the glossopharyngeal nerve. Above these nerve fibers, the stimuli can be transferred from a number of

general sensory fibers of the trigeminal nerve and lay in the lingual epithelium. It is obvious that the boundary between the area supplied by the lingual nerve and the chorda tympani cannot be easily discriminated. We must mention the participation of olfaction in the initiation of taste senses. This gustatory olfaction is caused by the pass of the olfactory substances of the foods through the olfactory cleft when someone expires during mastication. Obviously olfaction can cause the initiation of taste stimuli and appetite, but can also cause the depression of it.

Taste transduction

The main role of the taste receptors is to transform the chemical signals into changes of the potential of the membrane the intracellular concentration of calcium ions. These mechanisms have a great variety in number and pathways in every type of neuron.

Taste disorders

The disorders of taste are very common and can be caused by many factors such as:

- Lesions at the mucosa, taste buds and impairment of the cranial nerves can cause taste disorders.
- Oral inflammation and mucositis can impair taste sensation.
- Malignancies and tumors of head and neck cause inability to appreciate flavours.
- Endocrine disorders also are involved in taste and olfactory disorders. Diabetes mellitus and hypothyroidism lead to changes of taste sensitivity.
- Drug toxicity caused by substances such as acetylsalic acid, biguanidune, carbamazepine, levodopa, ethambutal gold, penicillamine and lithium. Drugs damage the sensory endings or the peripheral nerves.
- Other causes are hereditary disorders (aplasia of the taste buds), consumption of alcohol, radiotherapy and chemotherapy, lack of copper and vitamin A.

Evaluation of taste

Taste is evaluated with two methods: the chemical gustometry and Electrogustometry. According to the first method the clinical doctor applies substances, which represent the four taste qualities of sweet, salty, sour and bitter in different concentrations in order to determine the lowest concentration that can be recognised.

When Electrogustometry is used, then the clinical doctor uses electric current to stimulate the taste receptors. An anode current is used ranging between 2 and 8 mA. This method is, in addition to chemical gustometry, quantitive and not qualitative. It is necessary to evaluate the function of the chorda tympani (**1-6**).

2. CASE REPORT

A 27-years-old male healthy volunteer was referred to our department and his magnetoencephalography (MEG) was evaluated in the following three states: physiological condition; after he ate sweet; after he ate salt.

Biomagnetic measurements were performed using a whole-head Neuromag 122-channel MEG system in a magnetically shielded room of low magnetic noise with broadband (f>10Hz) gradient noise 5fT/ (cm √Hz) for the 95% of the channels and max noise 10fT/ (cm √Hz), a broadband (1Hz<f<10Hz) gradient noise 15fT/ (cm √ Hz) for the 95% of the channels and max noise 20fT (cm √ Hz) (**7, 8**). The pick-up-coils of the device are shaped like figure-of-eights to make them "near-sighted", i.e. sensitive to sources in the brain, but insensitive to the ambient noise fields. The device employs planar gradiometers, which record at each of the 61 measurement sites, the magnetic field component normal to the helmet-shaped dewar bottom surface.

During the recordings the subject was sitting in a chair with his head covered by the helmet-shaped dewar. A head position indicator with three small coils was fixed on the scalp. The spontaneous MEG recordings were obtained with a sampling frequency of 256Hz and filtered with cut – off frequencies between 0.3 to 40Hz. The time taken for each recording was 5 min.

There was a differentiation in the spatial distribution of the frequencies over his head in the three states after the application of Fast Fourier Transform in his MEG data (**Figs 1-3**).

Figure 1. Spontaneous MEG activity recorded with the 122-channel planar gradiometer(t (220ms ,495ms), magnetic field (-584.5, 584.5 fT /cm)).

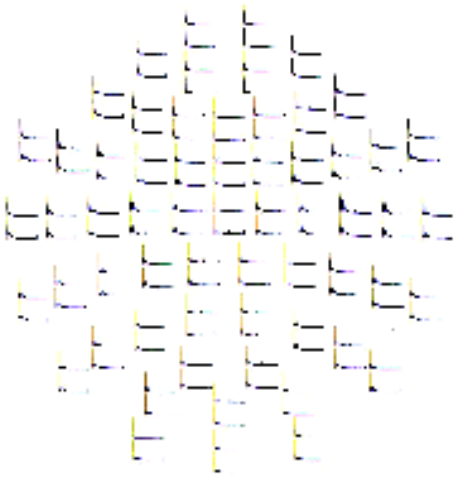

Figure 2. The FFT transform of the spontaneous MEG activity of Fig.1 (frequency range 1-20 Hz)

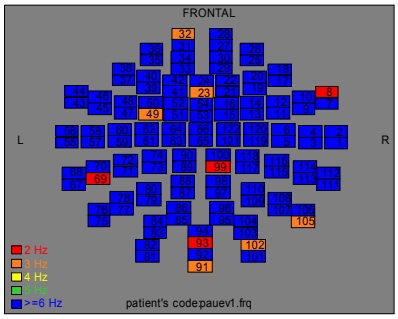

Figure 3. The frequency distribution of each channel over the scalp in the physiological condition

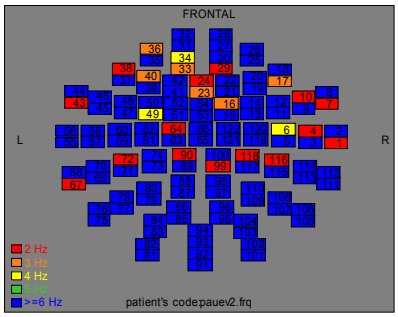

Figure 4. The frequency distribution of each channel over the scalp with a sweet taste

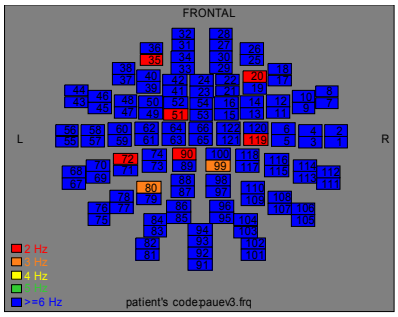

Figure 5. The frequency distribution of each channel over the scalp with a salt taste

3.CONCLUSIONS

The cerebral cortex is known to produce weak magnetic fields that can be recorded using the Superconducting Quantum Interference Device (SQUID) [7,8]. Such measurements are known as magnetoencephalogram (MEG).

Ionic movements throughout the neuronal cell body creating a current dipole follow changes in membrane potential. The orientation of the current dipole is a critical factor, which affects the measurement of magnetic fields. The MEG produced by such fields is exclusively created by a flow of electric currents tangential to the skull surface and therefore the signal will originate maximally from the cerebral sulci (where the pyramidal cells are more favorably oriented) and only minimally from the gyri surface where their orientation is less favorable [8].

Time and frequency – domain analysis of neuromagnetic data give complementary information about the underlying neuronal generators. In general analysis in the time domain is appropriate for transient behavior, and the frequency – domain analysis for rhythmic activity. Source localization algorithms may be applied formally for data in either domain. The lack of new methods, which can be used to evaluate taste, forced the authors to use the 122-channel biomagnetometer SQUID. Our results provide useful insights into the connection of taste with the CNS encouraging more studies to be conducted.

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Non Linear Analysis of SQUID Signals in Patients with Malignant Brain Lesions. Can Chaos Detect Cancer

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Abstract: - Non linear signal analysis is a powerful technique that reveals qualitative and quantitative differentiations between different dynamical systems (biological or otherwise). Presented here is a work in progress the goal of which is to investigate the differentiation of Magnetoencephalograms (MEG) received from patients with malignant CNS lesions and from healthy volunteers. Furthermore, explored are the differentiations of signals from patients undergoing various treatment schemes for the aforementioned lesions.

We present MEG recordings of 10 patients diagnosed with malignant CNS lesions and the corresponding ones from 10 healthy volunteers. Additionally we present follow up recordings on two cases, one of which was undergoing chemotherapy and the other radiotherapy A 122-channel SQUID biomagnetometer in an electromagnetically shielded room was used to record the MEG signals and the Grassberger-Procaccia method for the estimation of the correlation dimension was applied on the phase space reconstruction of the recorded signal from each patient.

Evidence linking the existence of low dimensionality chaotic dynamics with the existence of the tumour was found from this analysis.The obtained results substantiate our hypothesis of a relation between tumours of the brain and the chaotic nature of the neural dynamics derived from their MEG recordings.

Key-Words: MEG, Chaos dynamics, non linear analysis, brain tumors.

1. Introduction

Non linear analysis has been used in the past to assess magnetoencephalographic recordings from patients with schizophrenia [1] and Parkinson's disease [2]. This method was also used in the assessment of neonatal magneto-encephalography [3]. With this paper we present evidence pointing to a clear link between the existence of a tumour in the brain and the qualitative characteristics of the strange attractor derived from the Magnetoencephalographic recordings received from it with the use of non-linear methods of analysis.

MEG magnetic activity is caused by ionic movements across the plasma membrane [4]. This activity although exceedingly weak (~ 10^{-8} of the earth's magnetic field which is equivalent to 50 μT), can be measured by means of a Superconducting the statistical nature of neuronal discharges, is a very high, theoretically infinite complexity system [8].

A malignant lesion of the brain, on the other hand, has an important electrophysiological characteristic of high organization with constant and high ionic Quantum Interference Device (SQUID) [4]. The SQUID is a diagnostic tool capable of measuring the exceedingly weak magnetic fields emitted by living tissues. The higher the concentration of living cells in the test area, the higher the biomagnetic fields produced and recorded from it [5]. The method is non-invasive because the SQUID is a passive receiver and not a transmitter [6].

Chaos theory [7] provides us with measurable quantities of the complexity of dynamical systems. In the case of MEG time series these measurable quantities refer to the underlying dynamical system, namely the brain. Thus chaos theory, as applied in the MEG signal analysis, provides us measurable quantities of the dynamic complexity of the brain. There are indications that the healthy brain, due to activity due to the rapid reproductive rate of the lesion's cells.

The hypothesis that the brain is a dynamical system of very high complexity and that the malignancies of the brain are a low complexity-high organization

system, the chaos hypothesis of brain dynamics, is explored in the present work. Specifically we apply the method of Grassberger and Proccacia on MEG signals derived from patients with brain tumours in various stages of treatment as well as on the corresponding signals of healthy volunteers. The purpose of this study is to explore the diagnostic potential of this method in the case of brain tumors as well as the potential use of this method as a treatment monitoring method.

2. Methods

Magnetic recordings were obtained from 10 patients with malignant lesions of the CNS and 10 healthy volunteers

The method used for the recording of magnetic activity has been described in detail elsewhere [6, 9,10]. In brief, we used a 122-channel SQUID gradiometer device and specifically the Neuromag-122 (Neuromag Ltd. Helsinki Finland). The 122 orthogonal thin-film planar gradiometers operate at low liquid helium temperatures (4o K) on the basis of the Josephson effect of superconductivity [11] with a broadband (f>10Hz) gradient noise 5 fT/(cm/$\sqrt{Hz}$) for the 95% of the channels and max noise 10 fT/(cm/$\sqrt{Hz}$) and a broadband (1Hz<f<10Hz) gradient noise 15 fT/(cm/$\sqrt{Hz}$) for the 95% of the channels and max noise 20 fT/(cm/$\sqrt{Hz}$). Recordings were taken in an electromagnetically shielded room in order to avoid extraneous electromagnetic noise. All 122 channels were examined for both the patients and volunteers. Recordings of duration in the range of 10-15 sec, with a sampling rate of 256Hz each, were taken. The duration of the above recordings is justified because the chosen time interval is enough to cancel out, on the average, all random events and to remain only the persistent ones.

2.1 Non-Linear analysis of MEG signals

Nonlinear analysis is a powerful technique for the estimation of the fractal dimension of the strange attractor that characterizes the magnetoencephalogram (MEG) time series obtained from patients with CNS malignant lesions. For the estimation of the dimension of the strange attractor we have considered the method proposed by Grassberger and Procaccia [12,13], which is based on the theorem of the reconstruction of the phase space introduced by Takens [14].According to that method, the dynamics of the system under consideration can be experimentally reconstructed from the observed time series of a single observable dynamic component, as it is in our case, the MEG. We also take into consideration Theilert's correction [15] by rejecting the k closest neighbors, that are temporally but not dynamically correlated. Thus for the discrete time series $B_i=B(t_i)$ (i=1,2...N) of the MEG, which is measured experimentally, the vector construction of V_i is given by the following equation:

$$V_i=\{B_i,B_{i+(k+1)\tau},...,B_{i+(m-1)(k+1)\tau}\} \quad (1)$$

This equation gives a smooth embedding of the dynamics in a m-dimensional space, and the resulting phase trajectory in the phase space, is topological equivalent to the original phase space. The reconstruction time τ is a suitable delay parameter, which may be chosen arbitrary. In our study in order to ensure that all the points we sampled for the phase space reconstruction were dynamically and not temporally correlated, along with Theilert's rejection of the k closest neighbors we selected the delay parameter τ as the time delay required for the autocorrelation of the signal (Eq.2) to become zero for the first time (first zero crossing point selection).

$$r(\tau)=\frac{\sum_{t=1}^{N-k}[x(t+\tau)]x(t)}{\sum_{t=1}^{N-k}[x(t)]^2} \quad (2)$$

If the dynamics of the physical system is chaotic, the evolution of the system in the phase space, once transients die out, settles on a submanifold, which is a fractal set, the strange attractor. The concept of strange attractors is of a great importance in chaotic dynamics, since its existence or absence is related to the behavior of the system as chaotic or deterministic. If a strange attractor exists, it can be described by a geometrical parameter the correlation of fractal dimension D. This parameter is related to the number of variables required to define the space of the attractor within the phase space. According to the method, proposed by Grassberger and Procaccia [12,13], D can be estimated from an experimental time series by means of the correlation integrals C(r,m) defined as:

$$C(r,m)=\lim_{n\to\infty}\frac{2}{n(n-1)}\sum_{\substack{i=1\\ i\neq j}}^{n-1}\sum_{j=1+i}^{n}\Theta(r-|V_i-V_j|) \quad (3)$$

where Θ(u) is the Heaviside function defined as (Θ(u)=1 for u>0 and Θ(u)=0 for u≤0), m is the embedding dimension and n is the number of vectors constructed from a time series with N samples, given by the formula n=N-(m-1)τ .The correlation integral C(r,m) measures the spatial correlation of the points on the attractor and it is calculated for different values of r in the range from 0 to rmax , where rmax is the maximum possible distance of two random selected points of the attractor of the selected time series. The r_{max} is equal to $(m)^{1/2}$ (x_{max}-x_{min}), (assuming that x_{max} and x_{min} are the maximum and the minimum recorded values in the time series). For a chaotic system the correlation integrals should scale as C(r,m) ~ rD(m). Thus, the correlation dimension D of the attracting submanifold in the reconstruction phase space is given by :

$$D=\lim_{\substack{r\to 0\\ m\to\infty}}\frac{\partial(\ln C(r,m))}{\partial(\ln(r))} \quad (4)$$

In the case of a chaotic signal exhibiting a strange attractor, there is a saturation value, indicated as a plateau in a graph of these slopes $\frac{\partial(\ln C(r,m))}{\partial(\ln(r))}$ v's ln(r), and which remains constant, although the signal is embedded in successively higher-dimensioned phase spaces. The saturation value of the slopes, gives an estimation of the correlation dimension of the attractor.

Using the above-described method, the correlation dimension D of the recorded MEG signals was estimated for each case. The purpose of this estimation was to determine whether the existence of a brain tumour and the biological differentiation caused by the treatment can be correlated with the dynamics of the recorded signal

3. Results

Using the aforementioned method the correlation integrals were calculated according to (Eq.3) for all the patients and the volunteers. As it is shown in Table 1 the recordings of the normal subjects present no saturation for embedding dimensions m as high as m=24 (as high as our sample size permitted).On the contrary the recordings from the patients do present saturation plateaus which begin from varying but low embedding dimensions m. Considering even the worst case scenario that the signals of the healthy volunteers do presented saturation for m=25 an independent sample t-test revealed that the significance value for the Lavene independent samples t-test is p=0,007, thus non equal variance is assumed. In this case the 95% confidence interval of the difference is between 9,8-15,4 not containing zero and the two tailed significance of the null hypothesis (m_{norm}=m_{path}) p<0.001.This test, summarized in table 2, established that a statistically significant difference between the two samples exists :

m_{norm}-m_{path} >12.

Additionally we were able to do follow up recordings on two patients. The first underwent the first radiotherapy session administering a 3 Gy dose fraction to the tumor while the second underwent complete surgical removal of the tumor prior to the follow up recording, while the second. As summarized in Table 3 the first patient's signal showed a significant increase in the m_{minsat} after the first fraction of radiotherapy while the second patients signal after the surgical removal of the tumor did not present saturation plateau for embedding dimensions m as high as m=24 (as high as our sample size permitted)

Table 1

Patients			Normals	
ID#	m_{minsat}		ID#	m_{minsat}
1	11		1	≥25
2	16		2	≥25
3	8		3	≥25
4	22		4	≥25
5	11		5	≥25
6	12		6	≥25
7	12		7	≥25
8	11		8	≥25
9	10		9	≥25
10	11		10	≥25

Table 2

Levene's Test for Equality of Variances			t-test for Equality of Means				
	F	Sig.	t	Sig. (p)	Mean Dif.	Std. Dif.	95% Dif. interval

							Low	Up
Equal variances	9,13	,007	10,16	,000	12,6	1,24	9,99	15,2
Non equal variances			10,16	,000	12,6	1,24	9,79	15,4

Table 3

# patient	m_{minsat} pre treatment	m_{minsat} after treatment
1	11	18
2	8	≥25

4 . Discussion

The data presented in this study provide some interesting insights in the dynamics of the signals received from CNS malignancies and also suggest further avenues of research for use of this method. The non-linear analysis of the MEG recordings of CNS malignancies presents some unique challenges but also some unique opportunities.

The normal neuronal discharges are of a statistical nature and thus they present no saturation; their fractal dimension is infinite [8]. On the contrary the signals from the area of the tumor are more organized and have low dimensionality as it was made clear from our results. These results indicate a correlation between the existence of a tumour in the brain and the onset of saturation in the slopes of the correlation integrals of the strange attractor of the MEG signal received from it, thus indicating the existence of low dimensional chaotic dynamics in the aforementioned signals.

The infinite dimensionality of normal neuronal activity, though, can assist us in extracting useful results about the low dimensionality source that is the tumor. Without it the recordings received from the area of the tumor would present the same dynamical characteristics, the same saturation point for the same embedding dimension regardless of the treatment's progress since the only parameter that changes with the treatment is the active cell count. That fact although it has an impact on the absolute intensity of the magnetic signal emitted from the tumor does not impact the signal's dynamics and dimensionality. But when there are, two overlapping sources of activity, one with low dimensionality and the other with infinite, reducing the first's intensity with the treatment (and thus its contribution to the overall signal) will "force" the dimensionality of the overall signal to rise. That is, the saturation point should appear for higher embedding dimensions than in the pre-treatment signals. Our experimental data confirm the above hypothesis. The recordings of the patient who was submitted to surgical extraction of the malignancy presented no saturation point after treatment while those from the patient who was just beginning radiotherapy, presented post-treatment saturation albeit for higher embedding dimensions.

Certainly more research is required on a larger patient base in order to establish quantitative thresholds according to histological data, the tumor's size and depth and to possibly quantify the effect of the various treatment schemes on the m_{minsat} Nevertheless these results point to a possible use of the non linear analysis of MEG recordings as a diagnostic and a treatment monitoring tool.

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Biomagnetic Measurements of Iron Stores in Human Organs

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Abstract: - The standard quantitative measurement of iron stores method was required surgical or needle biopsy which is very discomfort and in some cases with significant risk to the subject. An alternative method is proposed using the biomagnetometer SQUID. With the use of the SQUID is providing an accurate and without risk quantitative method of iron measurement in liver, spleen and heart for adults and children suffering from Thalassemia. The above non-invasive biomagnetic method exploits the effects of magnetism and supercon ductivity. Thus, when an organ, such as the liver or the spleen is placed in a magnetic field, it will slightly distort the applied field. If the liver is normal or anemic, the applied magnetic field will be reduced slightly. On the other hand if the liver is iron overloaded, as is in the case of patients suffering from Thalassemia, the local applied magnetic field will be enhanced. Hence, the change in the detected magnetic field measured with the SQUID will be directly related to the iron concentration of the organ under investigation.

Key-Words: SQUID, MEG, Thalassemia, Liver, Spleen, Heart.

1. Introduction

Time varying electric currents, in wires or brain cells, all produce time varying magnetic fields (1). The detection and isolation of neuromagnetic signals was a challenging problem. Thus, these magnetic fields, which are very weak in order to be detected we need very sensitive and sophisticated devices. Such sophisticated devices are the ones which are based on the Josephson effect of superconductivity (2) and are called SQUID from the initials of the words (Supercoductive Quantum Interference Device).

The SQUID's becomes superconducting when their sensors are immersed in liquid helium contained in a large dewar. The liquid helium cools the SQUID's sensor to 40 Kelvin (-2690 C). The SQUID has the ability to detect magnetic fields of the order of 10^{-15}T (fT).The signal measured by the SQUID is a time varying voltage waveform that reflects local changes in the magnetic flux as a function of time. The higher the concentration of living cells in the area under study, the higher the biomagnetic fields produced and recorded from it (3). As a result the magnetic field measured with the SQUID is very useful technique for clinical use.

2. Material and Methods

Magnetic recordings will be obtained from the liver or the spleen of the patients suffering from Thalassemia. On the first visit, the patient information including name, age, height and weight is taken. The depth and shape of the liver or spleen is measured by ultrasound and will be stored into the patient data base. The patient is positioned on a special wooden bed directly beneath to the SQUID detector and a weak magnetic field of the order of 0-20mT will be generated within the body tissue by an external magnet. When the under investigation organ is placed in the applied external magnetic field it will slightly distort the applied field measured by the SQUID. If the organ is normal or anemic, the field with the SQUID will be reduced slightly. If on the other hand the organ is iron overloaded, the measured field with the SQUID will be enhanced. Hence, the change in the detected magnetic field measured with the SQUID will be directly related to the iron concentration of the organ under investigation. To minimize the body's contribution to the distortion in the magnetic field a small bag of water is placed between the SQUID detector and the skin above the organ.

3. Results and Discussion

Since the susceptibility of the organ under investigation is close to that of the water in the bag, the resultant SQUID measurement will be essentially that of the magnetized organ, which is within a uniform diamagnetic environment, and the only change detected by the SQUID will be due to the

organ itself. For higher accuracy, our computer software removes any contribution from the tissues next to the organ under investigation (skin, bone, muscle, fat, etc.). This consideration gives the iron concentration of the organ alone for all kind of subjects. The above results are preliminary and need further investigations.

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Magnetic Stimulation in Universalis Alopecia Areata: Clinical and Laboratory Findings

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Abstract. Magnetoencephalography measurements and external magnetic field have been used in the differential diagnosis and management of gynaecological and neurological entities. Exogenous magnetic stimulation and superconducting quantum interference device findings have been applied in the treatment of an alopecia universalis case (in comparison with three other control cases). An important hair regrowth appeared progressively from the second week till the fifteenth month of the therapeutic protocol. The indubitable superiority (comparatively to the control group) was noted. The blockage mechanisms of alopecia areata pathogenetic processes by exogenous magnetic field, have to be more searched in the future.

Key Words : -Superconducting quantum interference device (SQUID)
-Exogenous magnetic stimulation therapy
-Universalis alopecia areata

1. Indroduction

Our laboratory has already utilized (with clearly verified efficacy) the magnetoencephalogram findings and the external magnetic field (of low frequencies and intensities) therapeutic action, particularly in the following two indications:

(a) Decrease of the frequency and intensity of epileptic subjects symptomatology(1)

(b) Determination of differences (with diagnostic and prognostic value) concerning the biomagnetic activity between benign breast diseases on the one hand and malignant breast neoplasms on the other hand(2)

The absolute normal black hair regrowth in an epileptic 6 year-old female child with coexisted alopecia universalis, constituted the reason why we decided to apply our methods in other patients (non epileptic) suffering from alopecia universalis. Unfortunately we have no pictures of the scalp in the above case, before the magnetic stimulation treatment.

magnetic field) constituted our control group. The prementioned biologic, paraclinic and brain magnetic profile (showing low frequencies) evaluations have been done in these three control group patients as well. Three months topical treatment with clobetasol and methyl salicylate was applied in the first patient (a 13 year old female

The case reported below concerns a non epileptic female adolescent with alopecia universalis.

2. Patients and Methods

Our patient was a white female adolescent aged of 12 year-old, presented alopecia universalis from the age of 4 year-old. No treatment had been applied in this patient for two years at least, before admission. Haematological, biochemical, endocrinological, immunological investigations, brain computed tomography and brain magnetic profile (with superconducting quantum interference device) evaluation, have been done. Pineal gland calcification and low frequencies (2 to 3 Hz) in the power spectrum of the recorded magnetoencephalogram have been ascertained. Patient was included in therapeutic protocol with low intensity external magnetic field (5 sessions per week) and a progressive hair regrowth (particularly on the parietal and occipital regions of scalp) began to appear from the end of the first week of this management. Three other patients suffering from alopecia universalis (non treated by external subject), three months topical treatment with anthralin (0,5%) in the second one (a 18 year old female) whereas therapeutic abstention in the third one (a 23 year old male). Intralesional corticoid injections have been avoided, on account of the danger of skin atrophy or general corticotherapy indesirable effects in a child or lastly because of the

rare but extremely catastrophic retina emboli amaurosis.

3. Results

After thirty external magnetic field sessions, an important but diffuse hair regrowth, on the parietal and occipital region in particular (and much less on the frontal and temporal area) occurred. The power spectra frequencies obtained from the magnetoencephalograms were elevated on 4 Hz. The initially white hair became black, progressively. Afterwards, hair has been stagnating for one month. Management continuation at patient's home (for avoiding magnetic energy loss),

assured rebeginning of hair growth. After fourteen months treatment a very important multicentric black hair regrowth was noted on the whole scalp. The new power spectra of the recorded magnetoencephalograms showed higher frequencies (5 to 6 Hz). Patient's hair regrowth condition is characterized by constantly progressive improvement. During the whole therapeutic process no cutaneous or systemic adverse effect appeared. At the present time we cannot foresee in advance, the wholly requisite duration of treatment. About the three patients of our control group: A mild irritation was noted during the anthralin treatment, in the second patient. Magnetoencephalography recorded no modifications of frequency in all the three patients of the control group. No hair growth appeared in the third patient after fourteen months follow up. The first white hair appeared on the patietal and occipital area (six weeks after anthralin and eight weeks after dermocorticoid-methyl salicylate treatment beginning). This hair growth disappeared two months after the end of management (in the patient treated with dermocorticoid and methyl salicylate) and five months after the end of the anthralin treatment, in the second patient two patients for the present time) verified the superiority, the stability of efficacy and the safety of this management, in comparison with conventional treatments, as topical dermocorticoid (in association with topical methyl salicylate) or topical anthralin.

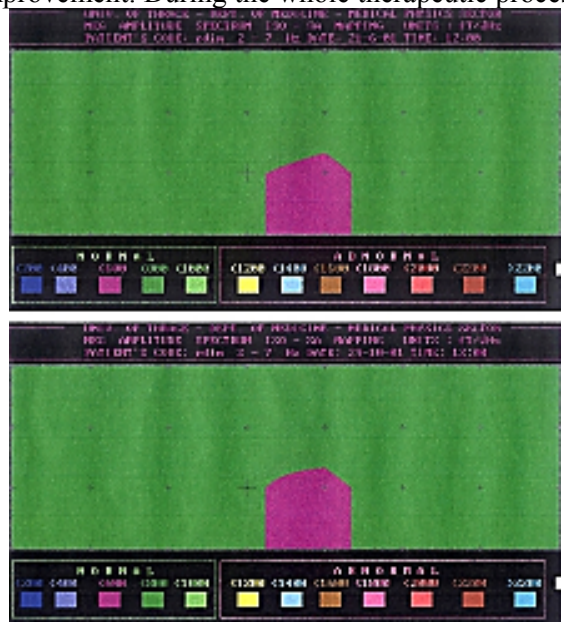

Fig.1 : The iso-spectra maps of left cerebral hemisphere (before and after treatment)

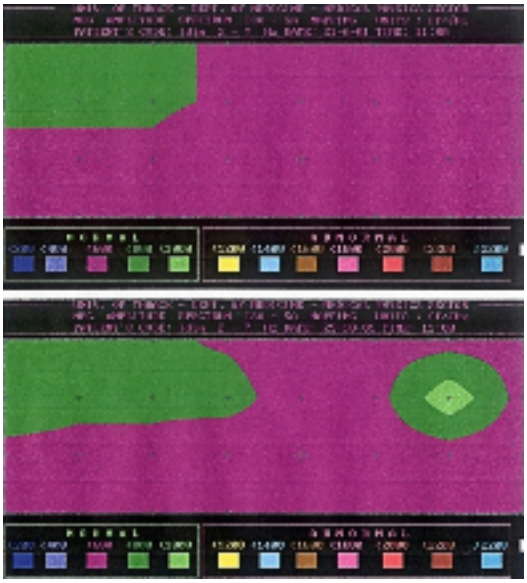

Fig.2 : The iso-spectra maps of right cerebral hemisphere (before and after treatment)

4. Conclusion

Universalis alopecia areata (also known as alopecia universalis) is a non cicatricial alopecia, concerning the totality of scalp hair, the eye-lashes, the eye-brows and the terminal hair of the androgen-dependent areas of the body. During the last 20 years, very important advances concerning profound pathogenetic comprehension of alopecia areata, have already been published. Nowadays, it is considered that a sudden passage from anagen stages (III or IV) to catagen or telogen stage, occurs. Various hypotheses (genetical, infectious, vascular, immune, neurologic, melanocytic, study of animal models) have been formulated in order to interpret the alopecia universalis

physiopathology(3)Disease repercussions on patient's psychosocial being are extremely unfavorable. Among the available therapeutic means for alopecia areata, it is worthwhile to be mentioned to photochemotherapy, topical application of detmocorticoids or minoxidil solution or vasodilative agents, contact allergen therapy, methylprednisolone intravenous administration sessions, anti-tumor necrosis factor-alpha antibodies intravenous administration sessions(4-7) Moreover there is a significant percentage of cases, characterized by spontaneous hair regrowth. The above presented alopecia universalis case constitutes the first one in the literature, which has been consciously treated with exogenous magnetic stimulation. External magnetic field therapeutic action (in patient's epileptic symptoms) could be attributed to alterations in the activity of the pineal gland, which in turn has been shown to regulate dopaminergic, 5-HT, GABA and endogenous opioid functions(1).

The successful external magnetic field therapeutic result (although it concerns only

ABBREVIATIONS

-GABA: γ-aminobutyric acid

- 5-HT: 5-hydroxytryptamine

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Multi-Channel MEG Evaluation of External Magnetic Stimulation on Parkinson Patients

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*Abstract.*Magnetoencephalogram (MEG) recordings of parkinson patients were obtained with the use of a 122-channel Superconductive Quantum Interference Device (SQUID), with which we have the ability for the whole-brain real-time monitoring and recording. The MEG signals were analyzed using linear and nonlinear signal analysis techniques such as Fourier Transform, distribution of MEG values, estimation of the autocorrelation of the signals and the correlation (fractal) dimension. In addition external magnetic stimulation (EMS) with magnetic field intensity in the pT range and α-rhythm frequencies was applied. The new MEG recordings shown a rapid attenuation of the abnormal MEG activity followed by a increase of the low frequencies components and of the α-rhythm after the application of the EMS. Further signal analysis findings indicated that the application of the EMS strongly influenced the underlying brain dynamics with beneficial effects on the clinical status of the parkinson patients.

Key words: MEG, Parkinson, EMS

1.Indroduction

Time varying electric currents, in wires or brain cells, all produce time-varying magnetic fields. Even though transmembrane, intracellular and extracellular neuronal currents each produce surrounding magnetic flux, the neuromagnetic field recordable outside of the head is a selective reflection of intracellular currents flowing in the apical dendrites of pyramidal cells oriented parallel to the skull surface. This is because of the biophysical properties of neuronal currents and the volume conduction properties of the head. The key to successful isolation of neuromagnetic signals is to use sensors that take advantage of how the strength of a magnetic field changes as a function of the distance from its source. Such magnetic fields emitted from the brain are very weak (of the order of $pT=10^{-12}$ T), so very sophisticated devices must be utilized in order to detect and record these fields. Such sophisticated device is the biomagnetometer SQUID (Superconductive Quantum Interference Device) which has the ability to detect magnetic fields of the order of 10^{-15} T (=1fT) [1]. The output of each channel of the biomagnetometer is a time varying voltage waveform that reflects local changes in the magnetic flux as a function of time. The appearance of the MEG signal is very similar to that of the electroencephalogram (EEG) and both normal spontaneous rhythms and pathologic activities are readily identified in MEG waveforms. Whereas MEG signals reflect current flow in the apical dendrites of pyramidal cells oriented tangential to the skull surface, EEG reflects both tangential and radial activity [2,3]. A key of locating the neuronal population that generates a neuromagnetic signal of interest is the adequate sampling of the spatial pattern of the magnetic field. This is most efficiently accomplished using a multichannel biomagnetometer system [4,5]. The most common model used for specifying the neuronal currents that generate a measured magnetic field pattern is that of a dipole in a sphere model. In this model it is assumed that the head can be modeled as a homogeneous spherical volume conductor and that the measured magnetic field pattern can be reasonably well characterized as though it were generated by an equivalent current dipole (ECD). Given the dipole-in-sphere model, it is possible to employ iterative computer algorithms to identify the location, orientation and strength of that dipole that best accounts (in a statistical sense) for the measured magnetic field pattern. The iterative procedure begins with the postulation of a current dipole at a particular position, orientation

and strength within the conductive volume. The Biot-Savart law is then used to forward calculate the magnetic signal that this hypothetical dipole would generate at each of the detectors [6]. At each detector, the difference between the forward-calculated signal and the actually measured signal is determined. The value of this mismatch term is then squared and summated across all of the detectors to generate an overall error term. The position, orientation and strength of this "best-fitting" dipole are then taken as indicative of the position, orientation and strength of the relevant neuronal currents. Although the importance of MEG recordings in the investigation of normal and pathological conditions of the brain (and especially in the study of epileptic phenomena), has been noticed by several authors [7,8], this methodology was also applied on Parkinson's diseased (PD) patients quite recently [9]. In these studies it was reported abnormal magnetic activity of well-localized regions of the cortex, which exhibited high amplitudes and rhythmicity with dominant frequencies in the range from 3 to 4 Hz. Furthermore, we applied also external magnetic stimulation (EMS) with proper characteristics (magnetic field in the order of pT and frequency the α-rhythm of the patient (8-13 Hz)) with the ones, which were obtained prior to the external stimulation [10]. The use of EMS has been used recently by several authors using transcranial and intracranial methodologies and it is proven to be a valuable tool for managing CNS disorders [11,13].
The patient's response to the EMS was a feeling of relaxation and partial or complete disappearance of muscular ache and levodopa induced dyskinesias as well as rapid reversed visuospatial impairment as

2. Material and methods

Patients and Recordings

PD patients were referred to the Laboratory of Medical Physics, by practicing neurologists. All patients had been diagnosed independently to suffer from idiopathic PD. Patients had normal routine serum biochemical studies, as well as, normal CT or MRI scans. In all cases informed consent for the methodology and the aim of the study was obtained from all patients prior to the procedure. The Hospital's Ethics Committee has approved this study. The MEG recordings were carried out in a magnetically shielded room with a whole head 122 SQUID (Superconducting Quantum Interference Device) gradiometer [16,17]. The pick-up-coils of the device are shaped like figure-of-eights to make

demonstrated by the Clock Drawing Test [9].This clinical improvement of the patients was followed by a corresponding improvement and normalization of the MEGs recorded after the application of EMS. Assuming that the MEG of PD patients is a reflection of the pathogenesis in the substantia nigra, dopaminergic functions and sympathetic ganglia, it appears that the application of the EMS has an immediate and beneficial effect on the dynamical condition of these pathological neural structures. On the other hand, the dynamics of any physical or biological system can be quantified and described by means of some new terms and concepts, such as the strange or chaotic attractor, the correlation dimension of the reconstructed phase space, the Lyapunov exponents and so on [14]. These concepts reflect some geometrical properties of the reconstructed phase space of the dynamical system under consideration and it is possible to be extracted, from recorded time-series of a single dynamical variable of the system, by the application of the appropriate algorithms. Of vital importance in the chaotic analysis of a dynamical system is the evidence for the existence of low dimension chaotic attractors and the estimation of the correlation dimension D of the attractor. In the present work the MEG time-series of the cortical magnetic activity of patients suffering from PD were recorded prior and after the application of EMS. In order to investigate for the existence of low dimensional strange attractors the Grassberger-Procaccia algorithm [15] was applied on the experimental time-series, aiming to establish a proper link of the observed alterations of the value of D, to the clinical improvements of the magnetic influenced PD patients.
them "near-sighted", i.e. sensitive to sources in the brain, but insensitive to the ambient noise fields. The device employs planar gradiometers, which record at each of the 61 measurement sites, the magnetic field component normal to the helmet-shaped dewar bottom surface. During the recordings the subject was sitting in a chair with his head covered by the helmet-shaped dewar. A head position indicator with three small coils was fixed on the scalp. The best-fitting ECD represents the location, orientation and intensity of current flow in the activated cortical source area. Typically, the whole field pattern could not be explained by one ECD and a two-dipole model was applied with one dipole located in each hemisphere. Initial guesses for the dipole locations were obtained from single-dipoles fitted to sets of channels in the neighborhood of the maximum signals. When the ECDs had been found, their locations and

orientations were kept fixed while their strengths were allowed to change to explain the whole epoch. This dipole-strength vs time curves served as indicators of the temporal behavior of the active areas. Power spectra were calculated with a frequency resolution of 0.3 Hz. External magnetic stimulation (EMS) was applied in the temporal lobes on the 32 - point matrix (4.5x10.5 cm rectangle) brain prior of the application of EMS. The time between the 1st MEG and post-stimulation MEG is about an hour. In all patients placebo tests were also performed. None of the patients experienced side effects during or after the procedure.

Data analysis

We applied nonlinear analysis in order to investigate the difference in the complexity underlying the dynamics characterizing the brain activity of normal and PD patients before and after magnetic stimulation.Nonlinear analysis is a powerful technique for the estimation of the dimension of the strange attractor characterizing the MEG time series obtained from the brain of PD patients. For the estimation of the dimension of the strange attractor we have considered the method proposed by Grassberger and Procaccia [15], which is based on the theorem introduced by Takens [19]. Then, according to their method, the dynamics of the system can experimentally reconstructed from the observed MEG time series $B_i=B(t_i)$ (i=1,2...N). Then, the vector construction of V_i is given by the following equation:

$$V_i=\{B_i,B_{i+\tau},...,B_{i+(m-1)\tau}\}$$

This equation gives a smooth embedding of the dynamics in a m-dimensional phase space. The evolution of the system in the phase space, once transients die out, settles on a submanifold, which is a fractal set, the strange attractor. The concept of strange attractors is of great importance in chaotic dynamics, since its existence or absence is related to the behavior of the system as chaotic or deterministic. The strange attractor can be described by a geometrical parameter, the correlation of fractal dimension D. This parameter is related to the number of variables required to define the attractor within the phase space and it can be estimated from an experimental time series by means of the correlation integrals C(r,m) defined as:

$$C(r,m)=\lim_{n\to\infty}(n(n-1)/2)^{-1}\sum_{i=1}^{n-1}\sum_{\substack{j=1+i\\ i\neq j}}^{n}\Theta(r-|V_i-V_j|)$$

Where Θ (u) is the Heaviside function defined as (Θ(u)=1 for u>0 and Θ(u)=0 for u≤ 0), m is the embedding dimension and n is the number of vectors constructed from a time series with N samples, given by the formula $n=N-(m-1)\tau$ (Here τ is a delay parameter which is equal to the first zero crossing of the autocorrelation time of the MEG signal). The correlation integral C(r,m) measures the spatial correlation of the points on the attractor and it is calculated for different values of r in the range from 0 to r_{max} . The $r_{max\ is}$ equal to $(m)^{1/2}$ $(x_{max}-x_{min})$, (assuming that x_{max} and x_{min} are the maximum and the minimum recorded values in the time series). For a chaotic system the correlation integrals should scale as $C(r,m) \sim r^{D(m)}$ .Thus, the correlation dimension D of the attracting submanifold in the reconstruction phase space is given by :

$$D=\lim_{\substack{r\to 0\\ m\to\infty}}\partial(\ln C(r,m))/\partial(\ln(r)) \quad (1)$$

(3)

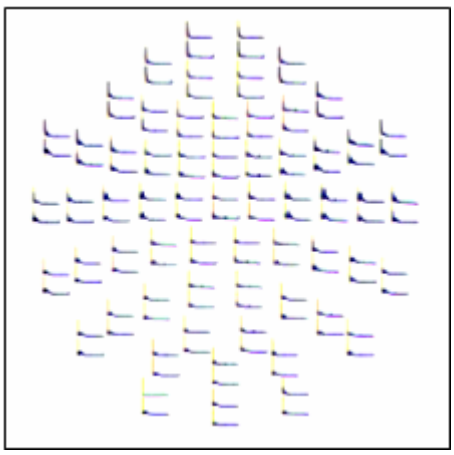
In the case of a chaotic signal exhibiting a strange attractor, there is a saturation value, (plateau) in the graph of the slopes ∂ (ln C(r,m))/ ∂ (ln(r)) v's ln(r). This value remains constant, although the signal is embedded in successively higher-dimensioned phase spaces and gives an estimation of the correlation dimension of the attractor. In the present work, the correlation dimension D of selected MEG time-series recorded prior and after the application of EMS were estimated, in order to detect and quantify any alteration on the brain dynamics of the PD patients due to the application of EMS.

3. Results

Fig.1A shows an example of spontaneous activity recorded with the 122-channel SQUID. The data were sampled at 256 Hz and filtered between 0.3

and 40 Hz. In figure 1B are presented the FFT transforms of the raw data of Fig.1A for each channel for the same time epoch whereas in Fig.1C we have the overlapping of the FFT transforms. Prominent low frequencies in the range of 2-7 Hz can be seen in the spectrum obtained from all channels whereas the alpha activities are either absent or very low (Fig.1C). Figure 2 shows the whole-scalp distribution of the magnetic field. The red colors show the outgoing whereas the blue colors the ingoing magnetic fields. The region where the color changes are placed the estimated ECDs.The above-discussed methods for measuring the brain dysfunctions in PD patients before and after the use of external magnetic stimulation have been tested in 30 patients aged 47 to 86 years (mean=69.1, SD=9.8). Table I gives the clinical response and individual data of every patient. In Figure 3A are shown the slopes of the correlation integrals from a PD patient in whom it is revealed the correlation dimension D (D=3.1) of the strange attractor prior to the application of the external magnetic stimulation. In the 30 PD patients that we have examined the mean value of D were 3.25 with standard deviation 0.31. In Figure 3B are shown the dimension correlation from the same patient after the magnetic stimulation in which it is revealed that the correlation dimension is shifted to higher values as it occurred in all the PD patients.If we compare Figure 3C which give the correlation dimension for normal subjects with the corresponding findings shown in Figures 3B we can see that we are dealing with a chaotic system. Such conclusion is very important because the application of magnetic stimulation provides a noninvasive method for managing patients with PD.

B



C

A

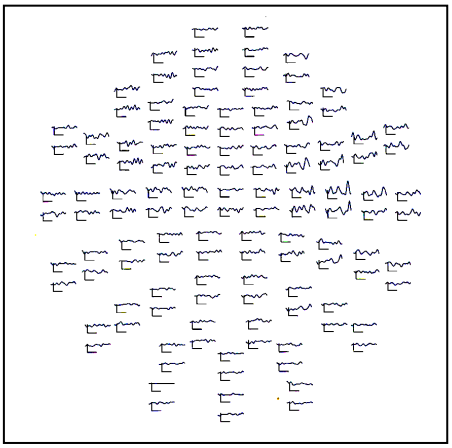


Fig.1. A) Spontaneous MEG activity, B) The FFT transform of the MEG activity, C) The overlapping FFTs from all channels

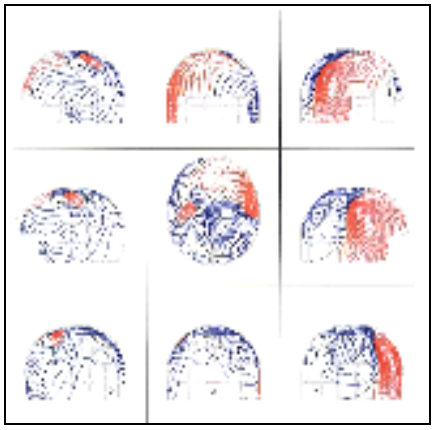


Fig.2. The MEG 3-dimensional mapping of the scalp for the same time period

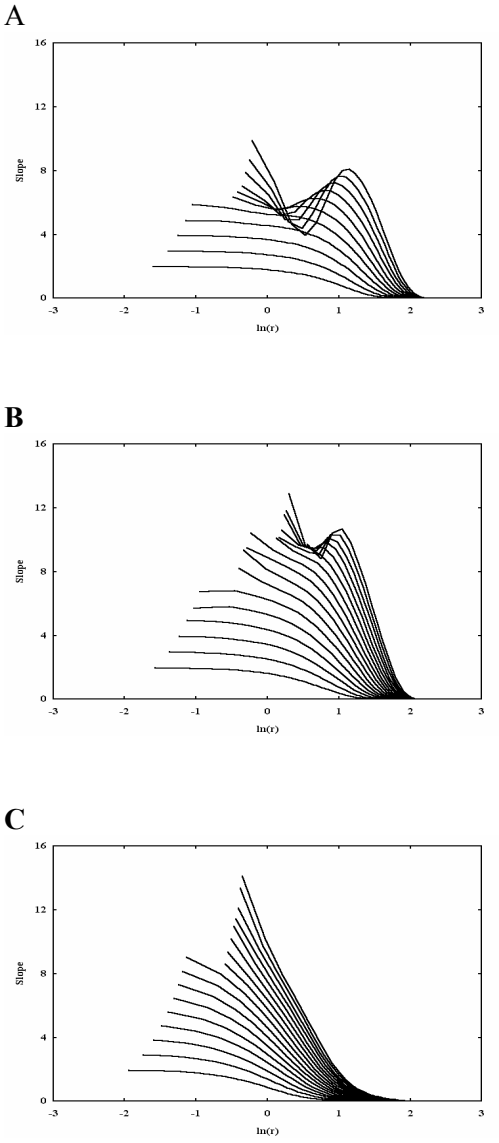


Fig. 3. Plots of the slopes of the correlation integrals from a PD patient A) before EMS, B) after EMS.C) . Plots of the slopes of the correlation integrals from a normal subject

SUBJECTS	AGE	AGE START	EEG DIAGBMS	EEG DIAGAMS	MEG DIAGBMS	MEG DIAGAMS	IMPROVEMENT (YEARS)	PLACEBO
MEN								
	62	52	P	N	A	N	2	NO EFFECT
	71	62	P	N	A	A	4	NO EFFECT
	68	52	N	N	A	N	3	NO EFFECT
	76	63	P	N	A	N	3	NO EFFECT
	74	67	P	N	A	A	3	NO EFFECT
	68	52	P	N	A	N	9	NO EFFECT
	62	45	P	N	A	A	2	NO EFFECT
	58	44	P	N	A	N	7	NO EFFECT
	86	73	P	P	A	N	6	NO EFFECT
	78	61	N	N	A	N	9	NO EFFECT
	68	59	P	N	A	A	1	NO EFFECT
	78	64	P	N	A	P	8	NO EFFECT
	79	65	P	N	A	N	8	NO EFFECT
	80	66	A	N	A	P	8	NO EFFECT
	76	55	N	N	A	N	9	NO EFFECT
	71	63	P	N	A	N	3	NO EFFECT
	56	45	N	N	A	N	8	NO EFFECT
	81	52	P	N	A	A	3	NO EFFECT
WOMEN								
	73	59	P	N	A	N	8	NO EFFECT
	49	34	P	N	A	N	3	NO EFFECT
	54	45	A	N	A	N	8	NO EFFECT
	47	42	P	N	A	N	2	NO EFFECT
	77	67	P	N	A	N	8	NO EFFECT
	80	62	A	N	A	P	8	NO EFFECT
	72	61	P	N	A	P	8	NO EFFECT
	65	50	P	P	A	N	6	NO EFFECT
	65	50	P	N	A	N	6	NO EFFECT
	72	58	A	P	A	P	8	NO EFFECT
	61	49	P	N	N	N	7	NO EFFECT
	68	58	P	N	A	N	7	NO EFFECT

Table 1. Individual data on the PD patients (N=30).A: abnormal; P: partial normal; N: normal diagnosis; DIAGBMS: diagnosis before magnetic stimulation; DIAGAMS: diagnosis after magnetic stimulation

4. Discussion

Time and frequency – domain analysis of neuromagnetic data give complementary information about the underlying neuronal generators. In general analysis in the time domain is appropriate for transient behavior, and the frequency – domain analysis for rhythmic activity. Source localization algorithms may be applied formally for data in either domain. However, the application of a specific

inversion algorithm, such as the ECD model, may or may not result in a useful inversion of the data. The utility of the algorithm depends on the goodness of fit between the structure of the sources in the model and the neuronal sources. In particular, frequency domain localization of ECD sources is useful if the underlying neuronal generators are differentiated harmonic content and spatial distribution. The existence of such sources has been demonstrated by the determination of ECD components of activity with both a sharp spectral peak and localized source volume. Both frequency and time domain analysis may be applied to the same epoch of time. The coincident occurrence of spike activity localization in the time domain and slow activity localization in the frequency domain may be an interesting tool for localization of epileptic activity. The separation of a complex set of sources underlying spontaneous activity into distinct components is an initial step in determining the functional significance of spontaneous activity.

Exposure of a biological organism or material to magnetic fields has been reported to induce a variety of effects which result to mutagenic, mitogenic, immunological, metabolic endocrine, behavioral and anticonvulsant [20,22] responses. It was also shown that EMS alter, at cellular level, the properties and stability of biological membranes as well as, their transport characteristics including the intra- and extra cellular distributions and flux of calcium ions [21]. Application of EMS on the brain of PD patients resulted in a rapid attenuation of Parkinson disability and partial or complete resolution of the levodopa-induced dyskinesias, which is a common side - effect complication of chronic dopaminergic therapy [9,23]. Although the striking beneficial effects of the application of the EMS on the clinical picture of the PD patients are well observed, the mode of action of EMS in PD remains an open question. This question is difficult to be answered given the complexity of cellular, systemic and neuroendocrine effects of EMS on biological systems. Our nonlinear techniques referred to the reconstruction of the phase space from the MEG time-series of the system, the detection of the existence of strange attractor and the estimation of its correlation dimension. Furthermore, dimensionality calculations (i.e. calculation of the correlation dimension of the strange attractor in the reconstructed phase space) can be utilized in the case of experimental biomedical signals, as is the case of MEG for the quantification of the complexity of the neuronal system under consideration. The results of the dimensionality calculations of different conditions of the dynamical system must be read in a rather comparative manner, due to the inclusion of external and/or inherent noise in the recorded signals. One additional reason which supports the comparative reading of the dimensional analysis results, is that in the case of brain signals like the MEG recordings, the obtained experimental time-series depends on the functionality of the brain, which exhibits characteristic peculiarities and individual behavior for each subject. This effect was also shown in the case of nonlinear EEG signal analysis [24].

In all PD patients that we have examined, we have observed a shift in its corresponding correlation dimension in the MEG recordings after the external magnetic stimulation, which is also related to the substantial clinical relief of the patients from the above-described physical and functional disabilities. In terms of the pathophysiology of the PD, the observed increased complexity, which appeared in the slopes of the correlation integrals of the MEG activity in PD patients after magnetic stimulation, can be expressed as a distortion of the high rhythmicity, abnormal synchronization and coherence of neural activity. Such non-deterministic behavior of the MEG in PD patients after the application of external magnetic stimulation is comparable to the slopes of the correlation integrals of the MEG signal obtained from a normal subject (Figures 3B,C). The PD patients before and after magnetic stimulation were in physiological state without any signs of drowsiness or alertness. The EMS stimulation that we have applied is so weak that the patients don't feel anything

during the application but they feel relaxation after it, which means that the changes that we notice are not only in the MEG measurements.Thus, this method of magnetic stimulation may be considered very important, non-invasive modality in the management of idiopathic PD patients. Magnetoencephalogram (MEG) recordings of parkinson (PD) patients were obtained with the use of a 122-channel Superconductive Quantum Interference Device (SQUID), which gave us the ability for whole-brain real-time monitoring and recording. The MEG signals were analyzed using linear and nonlinear signal analysis techniques such as Fourier Transform, distribution of MEG values, estimation of the autocorrelation of the signals and the correlation dimension (D). In the 30 PD patients that we have examined the mean value of D was 3.25±0.31. On the above patients external magnetic stimulation (EMS) was applied with proper characteristics (magnetic field in the order of pT and frequency the α-rhythm of the patient (8-13 Hz)) with the ones, which were obtained prior to the external stimulation. MEG recordings shown a rapid attenuation of the abnormal MEG activity followed by a increase of the low frequencies components and of the α-rhythm after the application of the EMS. The patient's response to the EMS was a feeling of relaxation and partial or complete disappearance of muscular ache and levodopa induced dyskinesias as well as rapid reversed visuospatial impairment. This clinical improvement of the patients was followed by a corresponding improvement and normalization of the MEGs recorded after the application of EMS. Furthermore, dimensionality calculations are utilized in the case of experimental biomedical signals, as is the case of MEG for the quantification of the complexity of the neuronal system under consideration. One additional reason which supports the comparative reading of the dimensional analysis results, is that in the case of brain signals like the MEG recordings, the obtained experimental time-series depends on the functionality of the brain, which exhibits characteristic peculiarities and individual behavior for each subject. Thus, the EMS application may be considered as a non-invasive modality in the management of idiopathic PD patients.

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Evaluation of an Intracranial Arachnoid Cyst with MEG after External Magnetic Stimulation

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*Abstract.*Purpose: To assess the utility of the magnetoencephalogram (MEG) as a non-invasive imaging modality in the diagnostic evaluation of a patient harbouring an intracranial arachnoid cyst, after external magnetic stimulation (EMS).Materials and Methods: A 34-year-old male diagnosed of an intracranial arachnoid cyst arising in the left-sided temporal-parietal area, by computed tomography (CT) underwent evaluation by means of MEG. Biomagnetic waveform recordings were obtained from the target area and the Fourier analysis of these measurements was carried out. External magnetic stimulation (EMS) with proper field characteristics (intensity: 1-7.5 pT, frequency: 8-13 Hz) was applied and the emitted MEG activity was recorded again.
Results: The cortical area adjacent to the borders of the arachnoid cyst emitted biomagnetic waveforms with high values. The application of EMS resulted in a rapid attenuation of the high MEG activity in the target area.
Conclusion: MEG may provide useful information in the diagnostic evaluation of arachnoid cyst patients and could be a supplement to other imaging modalities. The lower activity and the relief from seizures after the application of EMS support the beneficial effects of magnetic stimulation in arachnoid cyst patients.

Keywords: Intracranial arachnoid cyst, MEG, external magnetic stimulation

1. Introduction

Arachnoid cysts are non-tumorous intra-arachnoid fluid collections that account for about 1% of all intracranial space-occupying lesions. They may develop throughout the cerebrospinal axis, with predominance in the sylvian region. Because of their benign nature and slow expansion, arachnoid cysts may usually remain asymptomatic or produce only subtle symptoms and signs. In a minority of cases, however, focal neurological deficits, raised intracranial pressure, and/or epileptic seizures may develop. Therefore, the question of when these lesions should be operated upon is not always easy to answer. Moreover, the choice of the most appropriate surgical approach remains debatable. (**1**)Thanks to new technical developments, functional mapping of the human brain has made tremendous progress in the past years. Single photon emission computer tomography (SPECT), positron emission tomography (PET), functional magnetic resonance imaging (fMRI) and high-resolution electro- and magnetoencephalography (EEG and MEG) are current techniques intensively applied to functional studies, each one having specific advantages and disadvantages concerning spatial and temporal resolution. The success of these methods in basic neuroscience research has created a demand for applying them to clinical questions. Diseases of the central nervous system that lead to brain dysfunction can be ideally explored using these techniques. Of particular importance are those diseases in which a focal neuronal dysfunction is the primary cause and where surgical resection of this focus might be the cure. This is often the case of tumors associated with pathological rhythms development and spread throughout the brain, leading to seizures that severely handicap the patient. Surgical resection of the primary focus is only possible if the focus can be precisely localized and adequately separated from functionally important areas. The only methods that have sufficient temporal resolution to follow neuronal activity in real time are the electrophysiological measures, i.e. the EEG and the MEG (**2**).
This study investigated the utility of MEG and the effects of external magnetic stimulation in the assessment of an intracranial arachnoid cyst.

2. Case report

A 34-year-old male with recent onset of headaches and seizures underwent a CT scan. An 8cm in diameter arachnoid cyst arising in the left temporal-parietal region was disclosed. Subsequently, the

patient was evaluated with MEG. Biomagnetic waveform recordings were obtained from the target area and the Fourier analysis of these measurements was carried out. Informed consent was obtained from the patient prior to the procedure.

Biomagnetic activity was recorded using a single channel second order gradiometer SQUID (model 601 of the Biomagnetic Technologies Inc., San Diego, USA) which was located in an electrically shielded room. The measurements consisted of data recorded from the scalp at specified points as defined by the International 10-20-electrode placement system (**3**). The MEGs were recorded from both the left and right temporal areas from a total of 64 positions. The spatial distribution of the amplitude of the MEG power spectrum was examined using computer-generated graphics and expressed in terms of the total average of isocontour spectral amplitude distribution (ISO-SA) on the surface of the scanned areas over the scalp. These maps were useful in obtaining clearly defined areas of high spectral density in the 2-7 Hz band frequencies. The isocontour lines correspond to equal spectral amplitudes for each frequency band. Different colors represent different spectral amplitudes for the same band frequency. Mapping the spectral power distribution over a surface requires that the recorded MEG activity remains invariant in time. In order to ensure the stability of MEG activity we repeated the recordings at various positions at different times and found that there was no difference in the power spectrum between recordings made as much as 60 min apart. Thus, the stability of MEG measurements in patients with CNS disorders justify the use of a one-channel SQUID (**4-6**).

External magnetic stimulation (EMS) was applied in the frontal, occipital and temporal lobes using an electronic device (**5,7,8**) and the emitted magnetic activity was recorded again. The electronic device consists of a low voltage generator, which can produce low frequencies from 2-13 Hz to a group of coils of 1 cm diameter. The 32 coils are enclosed between two parallel plane surfaces in such a way that the axis of the coils is situated perpendicular to these surfaces. They are situated on the 32-point matrix, which is defined previously. The applied EMS carried similar field characteristics (intensity: 1-7.5 pT and frequency (8-13 Hz)) with the ones emitted from the patient's brain prior to the application of EMS. The time between the 1st MEG and post-stimulation MEG was about an hour. After 5-months of treatment with EMS there was a significant reduction in the incidence of seizures.

Figure 1A shows the MEG map of the left temporal-parietal area of the patient with high biomagnetic values emitted from the arachnoid-cyst area due to the increased ionic movements from the pressure on the surrounding neural structures. The maximal total average emitted power in the 2-7 Hz band frequency was > 2200 Ft/ √Hz. **Figure 1B** shows the MEG map after 5 months treatment with EMS. There is a distribution of lower biomagnetic values in the cyst region. The maximum total average of spectral amplitudes emitted by normal subjects had measured values < 1000 Ft/ √Hz.

3. Discussion

Intracranial cerebrospinal fluid (CSF)-containing cysts may be caused by a variety of mechanisms, including trauma, hemorrhage, and inflammation. True arachnoid cysts are, however, widely accepted to be developmental anomalies in which splitting or duplication of the primitive arachnoid membrane in the early embryonal life leads to intra-arachnoid fluid collection. These lesions may remain small and clinically silent, or enlarge and produce symptoms mainly by virtue of their mass effect and pressure on the surrounding neural structures. The clinical manifestations of arachnoid cysts are variable and often unspecific. The most common presenting symptoms and signs are those of raised intracranial pressure, craniomegaly, and developmental delay. Focal neurological deficits and epilepsy are present in less than 30% of patients with middle fossa arachnoid cysts. A similar percentage of patients with suprasellar cysts may show visual impairment (**1**).

CT scan and MR imaging are diagnostic in the majority of cases, alleviating the need for histopathological confirmation. CT imaging features include a CSF density mass that effaces adjacent sulci and remodels bone. Small cysts may be undetected due to partial volume averaging, especially when located in the middle cranial fossa. Expansion of the sylvian fissure or bony scalloping may also indicate the presence of a cyst. On an MRI, the arachnoid cyst appears as an extra-axial mass that follows CSF intensity on all pulse sequences. Occasionally, the signal intensity of an arachnoid cyst can be similar or identical to that of an epidermoid tumor. Smooth erosion of the innertable of the skull is seen on both CT and MRI, which is thought to be the result of extremely slow growth and transmitted CSF pulsations. There is no enhancement (**9**).

Small cysts with minimal symptoms should be treated conservatively with regular clinical and

radiological follow-up examinations at six-month to one-year intervals. Large space-occupying cysts and those causing neurological impairment require surgical treatment. At present, cystoperitoneal shunting is the treatment of choice. Craniotomy and excision of the cyst should be reserved for those cases with recurrent shunt failure. The role of endoscopic cyst fenestration is still to be determined.

The data presented here, although preliminary, suggest that this method of measuring the magnetic activity of an intracranial arachnoid cyst can be potentially utilized in the follow up of patients (**Figure 1A,B**). This is not unexpected since the pressure of the cyst to the surrounding neurons produces magnetic fields of higher intensity than normal tissues. The possible mechanisms by which EMS has attenuated the patient's MEG activity is still controversial. However, one possible electrophysiological explanation for the efficacy of magnetic stimulation has been provided by the proposed "neural net model" (**10**), which suggests that magnetic stimulation causes a temporary neuronal inhibition in regions exhibiting paroxysmal discharges. The hypothesis is in accordance with data presented by other investigators. However, it is known that magnetic fields alter the activity of the pineal gland, which has been shown to regulate dopaminergic, and endogenous opioid functions (**11**). On a cellular level, the effects of magnetic fields on seizure activity may be related to alterations in properties and stability of biological membranes and their transport characteristics including their intra- and extra cellular distributions and flux of calcium ions (**12**). Another explanation for the management of epileptic activity using EMS is based on Morrell's hypothesis that every stimulus entering the brain is maintained for a certain period of time representing the short-term memory of the particular stimulus experience. (**13**) If the stimulus experience persists for an extended period of time then the short-term memory of the presented stimulus is converted to the permanent memory of the stimulus. Based on this neurophysiological principle it is possible to restore normal neurological activity in the brains of epileptic patients by the application of EMS of proper frequencies and intensities. In terms of pathophysiology, the distortion of the high rhythmicity or abnormal synchronization and coherence of neural activity which characterized brain activity is an indication that we are modulating activity in such a way that the characteristics of the time series are approaching the behavior of normal subjects (**5,7,8**). Our data imply that MEG recordings of an arachnoid cyst may prove useful as an adjunct to other imaging modalities by providing functional information in the evaluation of evolving disease. Additionally EMS shows inhibitory effects on the epileptic symptoms of these lesions.

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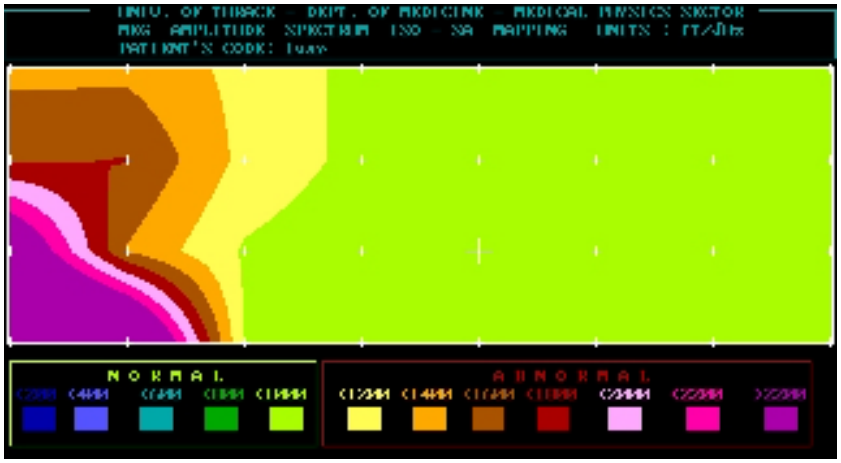
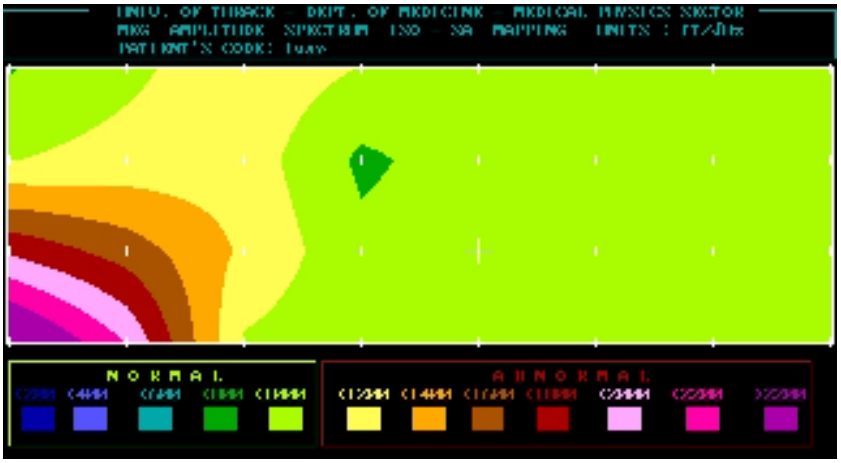
Figure Legends

Figure 1: A) The MEG map of the left temporal-parietal area of the patient with high biomagnetic values in the arachnoid-cyst region. B) The MEG map after 5-months treatment. There is a reduction in the MEG values in the cyst area.

A



B



MEG and MRI Evaluation in Parkinson Diseased Patients

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*Abstract.*Background: We investigated the localization of current sources for spontaneous MEG data in the frequency domain. MEG data are recorded as time-domain signals, which have been band pass filtered. The data were transformed from the time domain into the frequency domain by taking the fast Fourier transforms of each channel of data for some epoch. This procedure serves useful information about the spatial distribution of the underlying neuronal generators. Methods: The MEG recordings were carried out in a magnetically shielded room with a whole-head 122-channel magnetometer. Nine patients were examined suffering from Parkinson's Disease (PD). MRIs with T1-w and T2-w images were available in patient's records.Results: Time and frequency domain analyses may be usefully combined in the analysis of data sets that contain both transient and oscillatory components. Prominent low frequencies in the tremor range of 3-6 Hz can be seen in the spectrum obtained from all channels. MRI did not disclose specific findings in any case.Conclusions: Time and frequency domain analysis of neuromagnetic data gives complementary information about the underlying neuronal generators. In general, analysis in the time domain is appropriate for transient behaviour and in the frequency domain for rhythmic activity. It is suggested that the MEG could be a complementary method in the diagnostic evaluation of PD using spatial distribution of the raw data in the frequency domain.

Keywords: 122-channel magnetometer, Parkinson's disease, frequency domain, MRI

1. Introduction

Imaging modalities played an important role for evaluating the severity and progression of Parkinson's Disease (PD) and for the differentiation of PD from other neurodegenerative disorders involving the extrapyramidal motor system. Positron emission tomography (PET) and single photon emission computed tomography (SPECT) are modalities capable of providing an objective measure of PD severity in living humans as both techniques disclose the loss of neurotransmitter function (1). On the contrary, magnetic resonance imaging (MRI) has a limited diagnostic validity in PD, although non-specific abnormalities may be demonstrated on conventional MRI sequences. In advanced cases, atrophy of the substantia nigra is recognizable as a decrease in the width of the relatively hyperintense band seen in T2 weighted images of high field intensity and attributed to the pars compacta with a smudging towards the red nucleous of the anterior hypointensity due to deposition of iron in the pars reticulata (2). The classical domain of MRI is to differentiate the symptomatic parkinsonism from PD. Recent advances in MRI techniques demonstrated that it may be a valuable tool for detection of nigral pathology in PD and for differentiation of neurodegenerative disorders from atypical PD (1,3).

Non-invasive magnetoencephalographic (MEG) recordings identified involvement of deep diencephalic, pre-motor, primary motor and somatosensory areas in the tremor cycle of PD (4). The availability of MEG whole-head systems and methodological advances today allow investigation in more detail of the oscillatory network and mechanisms involved in PD tremor (5).

The goal of this study is to report the potential validity of MEG as a complementary method in the diagnostic work-up of PD using spatial distribution of MEG recordings in the frequency domain.

2. Methods and Results

Nine PD patients were referred to our laboratory by practicing neurologists. Patient's records included a routine MR imaging examination with axial and coronal T1-weghted images and T2-weighted images on three planes; i.e; axial, coronal and sagittal. Post gadolinium T1-weighted images were obtained from 2 out of 9 patients. Informed consent for the methodology and the aim of the study was obtained from all patients prior to the procedure.

Biomagnetic measurements were performed using a whole-head Neuromag 122 MEG system in a magnetically shielded room of low magnetic noise

(6-8). The pick-up-coils of the device are shaped like figure-of-eights to make them 'near-sighted', i.e. sensitive to sources in the brain, but insensitive to ambient noise fields. The device employs planar gradiometers that record at each of the 61 measurement sites the magnetic field component normal to the helmet-shaped dewar bottom surface. During the MEG recordings the individual sat on a chair with his head covered by the helmet-shaped dewar. All patients had minimal head tremor. Four indicator coils attached to the head of the individual subject determined the exact position of the head with respect to the MEG sensors. The positions of the coils were determined using a three dimensional digitizer. All MEG data were inspected carefully off-line for movement artifacts which were cut off from the data trace.

NO	SUBJECTS	AGE	MEG	MRI
	MEN			
1		74	abnormal	mild brain and cerebellar atrophy
2		64	abnormal	mild brain atrophy
3		56	abnormal	normal
4		54	abnormal	normal
	WOMEN			
1		55	abnormal	mild brain atrophy
2		69	abnormal	normal
3		60	abnormal	normal
4		56	abnormal	normal
5		60	abnormal	normal

Table 1. The Table 1 is showing N=9 subjects.

Figure 1 presents the first and second dominant frequency obtained from each channel.
The frequencies ≤1 Hz have been neglected. The red bars represent the abnormal frequencies in the range 1Hz < Fr ≤ 5 Hz.

Figure 1. The distribution of the first and second dominant frequency of the MEG power spectrum. The frequencies ≤1 Hz have been neglected. The red bars represent the abnormal frequencies ≤5 Hz.

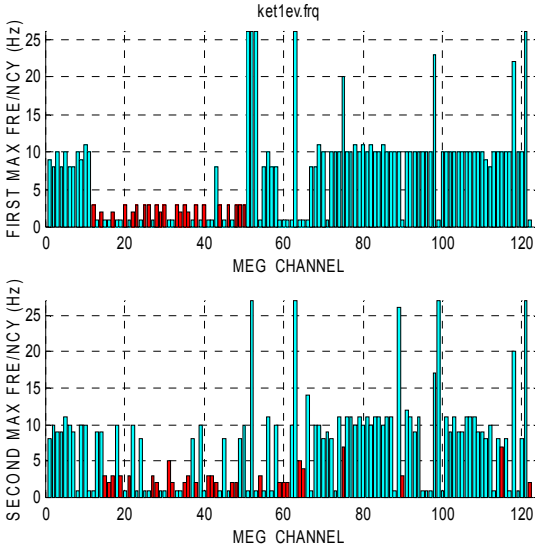

Figure 2. The spatial distribution of the first dominant frequency of each channel of fig.1 over the scalp. The different colors represent different frequencies.

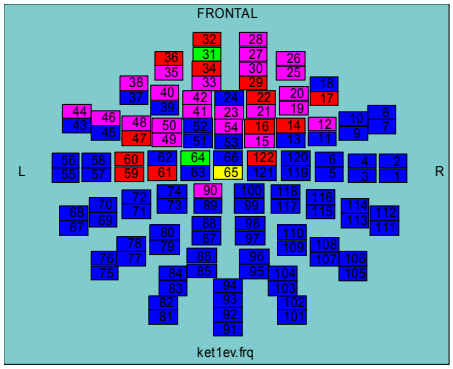

Figure 2 shows the spatial distribution of the first dominant frequency from one representative patient. When the first dominant frequency is ≤1 Hz, we chose the second dominant frequency. Different colors represent different frequencies.
MRI did not show basal ganglia abnormalities or specific focal parenchymal lesions. Mild brain atrophy was demonstrated in three patients associated with minimal cerebellar atrophy evident in one case.

3. Discussion

The brain is a complex dynamical system, so multichannel measurements are necessary to gain a detailed understanding of its behavior. Such multichannel measurements include optical brain images, multi-electrode recordings, functional magnetic resonance imaging, MEG, etc (8-10). In MEG, weak magnetic fields of the order of tens of fT/√Hz generated by electric currents in the brain are measured using superconducting quantum interferometric detector arrays positioned on the skull. The MEG is a noninvasive imaging technique, applicable to the human brain with

temporal resolution approximately ~1 ms (11). Several authors have demonstrated the importance of the MEG in the investigation of normal and pathological brain conditions during the last decade (8-14). These studies reported that the abnormal brain magnetic activity of well-localized regions of the cortex is in the striatum and in the basal ganglia, which are the motor parts of the brain and which are involved in the pathogenesis of neurological disorders of motor behavior including PD. The major advantage of MEG over electroencephalography (EEG) is that MEG has higher localization accuracy. This is due to the fact that the different structures of the head (brain, cerebrospinal fluids, skull and scalp) influence the magnetic fields less than the volume current flow that causes the EEG. Additionally, the MEG is reference free, so that the localization of sources with a given precision is easier for MEG than it is for EEG (15).

Clinical assessment cannot detect preclinical disease or reliably discriminate PD from atypical variants. PET and SPECT measurements of dopamine terminal function enable any preclinical dopaminergic dysfunction to be detected in at-risk relatives of patients with familiar disease or asymptomatic co-twins. Measurements of striatal function with PET and SPECT can help to discriminate with up to 80% specificity atypical parkinsonian syndromes from PD. ^{18}F-dopa PET and ^{123}I-β-CIT SPECT have been successfully used to monitor the rate of loss of dopamine terminal function (16).

MRI is a simple and inexpensive technique but its use in the diagnosis of PD is an elusive goal with a significant atrophy in the basal ganglia becoming obvious only in advanced disease stages. For the differential diagnosis of PD, the most important role of MRI is to differentiate the PD from symptomatic parkinsonism, pseudoparkinsonism and other degenerative diseases involving the dopaminergic system . The spectral analysis of multichannel data presents the fundamental problem of how to simultaneously visualize the time-frequency content of many channels. The abnormal PD activity exhibits high amplitudes and rhythmicity with dominant frequencies in the range from 3 to 6 Hz. Resting tremor in PD is a 3-6 Hz trembling, primarily affecting the distal portion of the upper limb. In advanced cases, the tremor may persist during movement or steady posture, whereas classically it is diminished or inhibited by voluntary action. There is increasing evidence that a central oscillator drives PD tremor independent of peripheral feedback. Volkmann et. al., (4) demonstrated using MEG that 3-6 Hz tremor in patients with idiopathic PD is accompanied by rhythmic subsequent electrical activation at the diencephalic level and in the lateral premotor, somatomotor and somatosensory cortex.Frequency analysis is being increasingly applied in the investigation of movement disorders. Thus far, studies have usually involved relatively small numbers of patients so that the degree to which findings may be true across large populations remains largely unclear (10). It seems, therefore, that there is a potential validity of MEG as a complimentary method in the diagnostic work-up of PD in the frequency domain. More studies are necessary before spectral analysis can provide an accessible and useful tool in the assessment of movement disorders in a routine clinical practice and as an adjunct to established diagnostic techniques.

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Measuring Brain Cancer through Chaos

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*Abstract.*Presented here are the results of a work in progress in order to investigate the differentiation of Magnetoencephalograms (MEG) received from patients with malignant CNS lesions and from healthy volunteers. We present MEG recordings of 10 patients diagnosed with malignant CNS lesions and from 10 healthy volunteers. A 122-channel SQUID biomagnetometer in an appropriate shielded room was used to record the MEG signals and the Grassberger-Procaccia method of phase space reconstruction was applied to the recorded signal from each patient.Clear evidence linking the existence of saturation plateaus with the existence of the tumour were found from this analysis further substantiating our hypothesis of a relation between tumours of the brain and the chaotic attractor derived from their MEG recordings. .

Keywords: biomagnetic activity, non-linear analysis, MEG

1. Introduction

With this paper we present evidence pointing to a clear link between the existence of a tumour in the brain and the qualitative characteristics of the strange attractor derived from the Magnetoencephalographic recordings received from it with the use of non-linear methods of analysis. MEG magnetic activity is caused by ionic movements across the plasma membrane (Rose et al. 1987). This activity although exceedingly weak (~ 10^{-8} of the earth's magnetic field which is equivalent to 50 μT), can be measured by means of a Superconducting Quantum Interference Device (SQUID) (1). The SQUID is a diagnostic tool capable of measuring the exceedingly weak magnetic fields emitted by living tissues. The higher the concentration of living cells in the test area, the higher the biomagnetic fields produced and recorded from it (2-4). The method is non-invasive because the SQUID is a passive receiver and not a transmitter.

2. Patients and Methods

Magnetic recordings were obtained from 10 patients with malignant lesions of the CNS and 10 healthy volunteers. All patients were diagnosed by medical experts to be suffering from cancer.
The Hospital Ethics Committee approved this study and informed consent was obtained from patients and volunteers prior to the procedure.

The method used for the recording of magnetic activity has been described in detail elsewhere (3-5). In brief, we used a 122-channel SQUID gradiometer device and specifically the Neuromag-122 of the 4D-Imaging company. The 122 orthogonal thin-film planar gradiometers operates at low liquid helium temperatures (4^o K) on the basis of the Josephson effect of superconductivity (6) with a broadband (f>10Hz) gradient noise 5 fT/(cm$\sqrt{Hz}$) for the 95% of the channels and max noise 10 fT/(cm$\sqrt{Hz}$) and a broadband (1Hz<f<10Hz) gradient noise 15 fT/(cm$\sqrt{Hz}$) for the 95% of the channels and max noise 20 fT/(cm$\sqrt{Hz}$). Recordings were taken in an electromagnetically shielded room in order to avoid ambient electromagnetic noise. From the 122-channels we recorded we selected for examination the channel nearest to the center of the malignancy for each patient as this was ascertained by the usual methods (CT or MRI scans). Recordings of duration in the range of 10-15 sec, with a sampling rate of 256Hz each, were taken. The duration of the above recordings is justified because the chosen time interval is enough to cancel out, on the average, all random events and to remain only the persistent ones.

Non-Linear analysis of MEG signals

Nonlinear analysis is a powerful technique for the estimation of the fractal dimension of the strange attractor that characterizes the magnetoencephalogram (MEG) time series obtained from patients with CNS malignant lesions. For the estimation of the dimension of the strange attractor we have considered the method proposed by Grassberger and Procaccia (7,8), which is based on the theorem of the reconstruction of the phase

space introduced by Takens (9).According to that method, the dynamics of the system under consideration can be experimentally reconstructed from the observed time series of a single observable dynamic component, as it is in our case, the MEG. We also take into consideration Theilert's correction (10) by rejecting the k closest neighbors, that are temporally but not dynamically correlated. Thus for the discrete time series $B_i=B(t_i)$ (i=1,2...N) of the MEG, which is measured experimentally, the vector construction of V_i is given by the following equation:

$$V_i=\{B_i,B_{i+(k+1)\tau},...,B_{i+(m-1)(k+1)\tau}\} \quad (1)$$

This equation gives a smooth embedding of the dynamics in a m-dimensional space, and the resulting phase trajectory in the phase space, is topological equivalent to the original phase space. The reconstruction time τ is a suitable delay parameter, which may be chosen arbitrary. If the dynamics of the physical system is chaotic, the evolution of the system in the phase space, once transients die out, settles on a submanifold, which is a fractal set, the strange attractor. The concept of strange attractors is of a great importance in chaotic dynamics, since its existence or absence is related to the behavior of the system as chaotic or deterministic. If a strange attractor exists, it can be described by a geometrical parameter the correlation of fractal dimension D. This parameter is related to the number of variables required to define the space of the attractor within the phase space. According to the method, proposed by Grassberger and Procaccia (7,8), D can be estimated from an experimental time series by means of the correlation integrals C(r,m) defined as:

$$C(r,m)=\lim_{n\to\infty}\frac{2}{n(n-1)}\sum_{\substack{i=1\\ i\neq j}}^{n-1}\sum_{j=1+i}^{n}\Theta(r-|V_i-V_j|)$$

where Θ(u) is the Heaviside function defined as (Θ(u)=1 for u>0 and Θ(u)=0 for u≤ 0), m is the embedding dimension and n is the number of vectors constructed from a time series with N samples, given by the formula $n=N-(m-1)\tau$ .The correlation integral C(r,m) measures the spatial correlation of the points on the attractor and it is calculated for different values of r in the range from 0 to r_{max} , where r_{max} is the maximum possible distance of two random selected points of the attractor of the selected time series. The r_{max} is equal to $(m)^{1/2}$ $(x_{max}-x_{min})$, (assuming that x_{max} and x_{min} are the maximum and the minimum recorded values in the time series). For a chaotic system the correlation integrals should scale as $C(r,m) \sim r^{D(m)}$ .Thus, the correlation dimension D of the attracting submanifold in the reconstruction phase space is given by :

$$D=\lim_{\substack{r\to 0\\ m\to\infty}}\frac{\partial(\ln C(r,m))}{\partial(\ln(r))}$$

In the case of a chaotic signal exhibiting a strange attractor, there is a saturation value, indicated as a plateau in a graph of these slopes $\frac{\partial(\ln C(r,m))}{\partial(\ln(r))}$ v's ln(r), and which remains constant, although the signal is embedded in successively higher-dimensioned phase spaces. The saturation value of the slopes, gives an estimation of the correlation dimension of the attractor.

Using the above-described method, the embedding dimension m above which saturation occurs was estimated for each patient or volunteer. The purpose of this estimation was to investigate whether the existence of a brain tumour can be correlated in the dynamics of the recorded signal.

3. Results

Using the aforementioned method we calculated the the embedding dimension m above which saturation occurs for each patient or volunteer. The results are presented in table1.

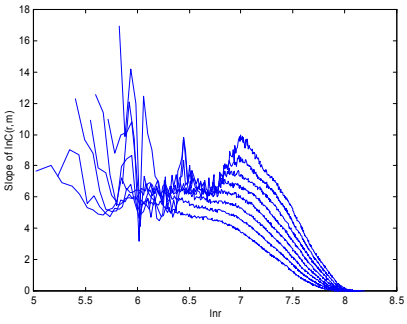

Figure 1. Typical graph of the slopes of the correlation integrals from a patient(m:7-14). Saturation present from m=10

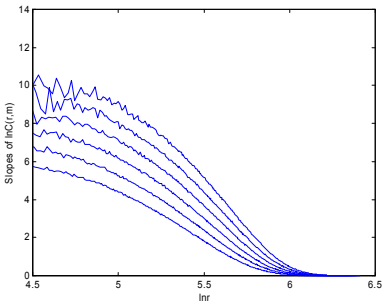

Figure 2. Typical graph of the slopes of the correlation integrals from healthy volunteer. No saturation present.

As it is shown in table 1 the recordings of the normal subjects present no saturation for arbitrarily high embedding dimensions m while the recordings from the patients do present saturation plateaus, beginning from varying embedding dimensions m .

Patients			**Normal subjects**	
ID no.	**m above which saturation occurs**		**ID no.**	**m above which saturation occurs**
1	**11**		*1*	∞
2	**16**		*2*	∞
3	**8**		*3*	∞
4	**22**		*4*	∞
5	**11**		*5*	∞
6	**12**		*6*	∞
7	**12**		*7*	∞
8	**11**		*8*	∞
9	**10**		*9*	∞
10	**11**		*10*	∞

Table 1

4. Discussion

The data presented in this study provide some interesting insights in the dynamics of the signals received from CNS malignancies and also suggest further avenues of research for use of this method. The non-linear analysis of the MEG recordings of CNS malignancies present some unique challenges but also some unique opportunities. The neuronal discharges are of a statistical nature and thus they present no saturation; their fractal dimension is infinite (11). On the contrary the tumour's signals are more organized and thus low dimensional as it was made clear from our results. This results opens new avenues for use of the non linear analysis of MEG recordings as a diagnostic tool.

Certainly more research is required on a larger patient base in order to quantify these so far qualitative results taking into account other important factors like histological data, the tumor's size and depth etc. but these results clearly indicate a correlation between the existence of a tumour in the brain and the onset of saturation in the slopes of the correlation integrals of the strange attractor of the MEG signal received from it

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Magnetic Field Profiles in Normal Human Breast During the Menstrual Cycle

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Abstract
The magnetic activity of normal breasts was recorded in four young women, aged 26 to 28 years, of whom two had regular and two prolonged menstrual cycles. The recordings were accomplished with the biomagnetometer SQUID, an instrument suitable of recording extremely small magnetic fields, and for every single case it covered two complete menstrual cycles on an, almost, everyday basis. The magnetic breast recordings in the two young women with presumed normal endometrial cycles showed a biphasic magnetic curve, corresponding to the proliferative and secretory phase of the menstrual cycle. By contrast, the two young women with prolonged menstrual cycles showed a monophasic magnetic curve. It is speculated that a biphasic, but not a monophasic, pattern of magnetic activity in the breast is suggestive of an ovulatory endometrial cycle.

Key words: biomagnetic fields, normal breast, menstrual cycle

1. Introduction

The female breast is a target tissue for sex steroid hormones and as such it responds to hormonal stimuli (1). These responses have been correlated with the menstrual cycle (2,3). Furthermore, the mammary gland, like all living tissues, emits magnetic fields produced by the continuous ionic movement across the plasma membranes (4). Interestingly, this activity, although very weak, can be recorded by means of a Superconducting Quantum Interference Device (SQUID).SQUID is a non-invasive research tool capable of recording the exceedingly weak biomagnetic signals generated spontaneously by all living tissues; it has been used successfully in recording breast activity in normal (5), benign and malignant breast tissues (6,7). In this study, we investigate whether the subtle histological changes observed in the normal breast tissues during the menstrual cycle can be recorded magnetically and correlated with the menstrual cycle of young women of the reproductive age.

2. Methods

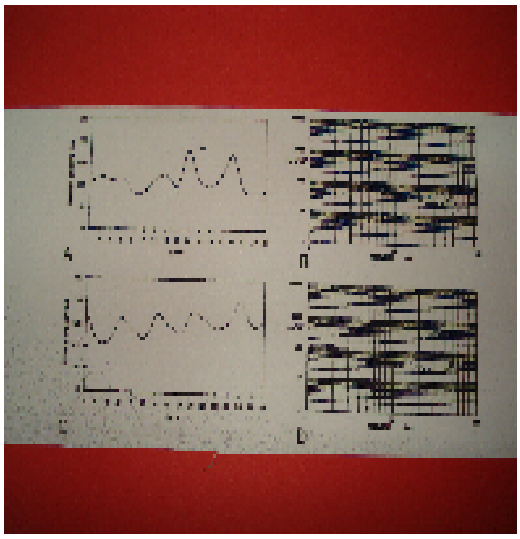
Four young healthy females, aged 24-28 years, were volunteered to take part in this investigation. Of these, two were married, had children and regular menstrual cycles, whilst the other two were single and had prolonged menstrual cycles. With the exception of the weekends, measurements were taken regularly on an everyday basis, starting from the first day of menstruation, and only occasional unexpected circumstances had resulted in the loss of some measurements. Informed consent for the study was obtained from all participating women prior to the procedure.The method used for recording magnetic activity has been described previously (5, 6, 8). In brief, we used a single channel SQUID with a sensitivity of 95 pTesla / Volt at 1000 Hz. (DC SQUID model 601, Biomagnetic Technologies, San Diego, USA). The gradiometer operates at the low liquid helium temperatures of 4^{o} K on the basis of the Josephson effect of superconductivity. In order to minimize interference from stray electromagnetic radiation, recordings were taken in an electrically shielded room of low magnetic noise, 400 meters away from the hospital. During the procedure the woman was lying supine on a wooden bed, free of any metallic objects. She was asked to relax and close her eyes to avoid artefacts from eye flickering. Recordings were taken from the upper / outer part quadrant of the right breast, i.e., the area with the largest proportion of lobular units (9), at a distance of 1 cm from the areola. For each point, 32 recordings of 1-second duration each were taken, with the SQUID detector placed 3 mm above the recording position. This would allow the maximum magnetic flux to pass through the coil with little deviation from the vertical direction. The duration of the recordings was adequate to cancel out all random events, leaving the persistent ones undisturbed. Only measurements in the frequency range between 2-7 Hz were considered. By convention, the maximum

value was used when assessing breast recordings. Data conversion of the analog signals into digital recordings was accomplished by means of an AD converter on line with a computer. The average spectral densities from the 32 1-second recordings were obtained after applying Fourier statistical analysis. In all cases, the signals were related to measurements of background magnetic activity (environmental magnetic noise).

3. Results

Figure 1A presents the biomagnetic profile in a young woman with presumed normal menstrual cycle. We observe high intensity waveforms generated by epithelial activity during the second half of the menstrual cycle. Figure 1B shows the spectral densities of a waveform from one representative day of figure 1 with a peak value of 110 fT/ √Hz at the frequency of 2 Hz. Figure 1C presents the biomagnetic profile in a young woman with prolonged menstrual cycle. We observe high intensity waveforms generated by epithelial activity throughout the menstrual cycle. Figure 1D shows the spectral densities of a waveform from one representative day of figure 3 with a peak value of 140 fT/ √Hz at the frequency of 2 Hz.

At first appreciation, it becomes evident that the magnetic fields varied from day to day from as low as 66 fT/ √Hz to as high as 179 fT/ √Hz with a mean value for all days of the cycle 115 fT/ √Hz. However, a more careful evaluation of the results shows that the young female volunteers with the presumed normal menstrual cycles have a biphasic magnetic curve, with low and almost flat spectral amplitudes during the proliferative phase of the cycle, and with 2-3 magnetic peaks during the secretory phase of the menstrual cycle (Figures 1A and 1B)



By contrast, the young females with the prolonged menstrual cycles showed a monophasic magnetic curve with low magnetic peaks uniformely distributed throughout the menstrual cycle (Figures 1C and 1D).

Figure 1A. Biomagnetic profile in young woman with presumed normal menstrual cycles: high intensity waveforms generated by epithelial activity are noted during the second half of the menstrual cycle.

Figure 1B. The power spectrum of the waveforms, after statistical Fourier analysis, from one representative day of figure 1. We observe a peak value of 110 fT/√Hz at the frequency of 2 Hz.

Figure 1C. Biomagnetic profile in young woman with prolonged menstrual cycle: high intensity waveforms generated by epithelial activity throughout the menstrual cycle.

Figure 1D. The power spectrum of the waveforms, after statistical Fourier analysis, from one representative day of figure 3. We observe a peak value of 140 fT/√Hz at the frequency of 2 Hz.

The plasma estrogen and progesterone levels of the four women under investigation are shown in Table 1.

Table 1.Blood sex steroid hormone measurements in the four women in the series during the menstrual cycle-case 1 and case 2 with normally cycling endometrium, case 3 and 4 with menstrual irregularities. The second measurements are shown in parentheses.

Case	Hormone	Day 7	Day 14	Day 21
1	Estradiol	75.8(67.1)pg/ml	421(435.7)	247.2(251.5)

	Progesterone	0.4(0.45)ng/ml	5.23(6.01)	19.2(17.5)
2	Estradiol Progesterone	160.1(145.7)pg/ml 0.2(0.39)ng/ml	592.2(610.3) 5.1(3.7)	210(230.4) 9.2(14.7)
3	Estradiol Progesterone	31.8(28.3)pg/ml 0.05(0.08)ng/ml	148.3(139.1) 1.45(1.43)	160.6(164.4) 2.5(2.1)
4	Estradiol Progesterone	42.6(34)pg/ml 0.04(0.03)ng/ml	110.9(121.1) 1.2(1.7)	80.7(125.5) 2.6(2.4)
Reference values.	Estradiol:proliferative phase 30.0-200.0pg/ml;	Ovulation 197.6-693.1pg/ml:	Sectetory phase 189.9-269.7pg/ml	Progesterone: Proliferative phase 0.06-0.65ng/ml: Ovulation 2.88-7.79 ng/ml;secretory phase 3.00-24.56.

4. Discussion

This study exploits the phenomenon of superconductivity to detect the subtle magnetic fields produced by the living normal breast tissue during the menstrual cycle. This was accomplished by means of a SQUID. Earlier studies, using light microscopical and electron microscopical techniques, have convincingly showed the existence of cyclic changes in the breast, following those of the endometrium (2, 3). In addition, differences in magnetic activity between normal, benign and malignant breast tissues have been detected by biomagnetometry (5, 6, 7). This is, however, the first time that such magnetic field changes are detected in normal mammary tissues during a menstrual cycle. More importantly, minor irregularities of breast responses to hormonal stimuli could be detected by this technique, reflecting analogous disturbances of the endometrial cycle. It is true that estrogen and progesterone receptors have been identified in both the endometrium and the breast, but it is now becoming apparent that the two-receptor systems share a common course in health and disease. It would be fair, therefore, to say that the biphasic magnetic curve noted in young women with presumed normal menstrual cycles is suggestive of an ovulatory endometrial cycle, while the monophasic magnetic field curve observed in young female volunteers with prolonged menstrual cycles may be indicative of a non-ovulatory endometrial cycle.

The higher magnetic fields noted in the second half of the breast cycle (secretory phase of the menstrual cycle) relative to first half (proliferative phase of the menstrual cycle) is in accord with the higher mitotic activity reported at this phase in the adult breast (1,3,10).

The implications of these observations are very early to be assessed, particularly when they are based on an extremely small sample, but may give a new dimension in the investigation of functional endometrial disturbances. Certainly, such an option would be encouraged by the harmless, non-invasiveness and the great precision of the method, which, in addition, is quick and easy to interpret. The cons of the method are, mainly, the unavailability of SQUID technology around the country and, perhaps, the impracticability of regular measurements.

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SUBJECT INDEX

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