THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

Quantitative EEG analyses in epilepsy patients

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The Examination takes place at Library of the Department of Anatomy, Histology and Embriology, Medical and Health Science Center, University of Debrecen
10.30. a.m. 26 April, 2011.

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The Ph.D. Defense takes place at the Lecture Hall of the 1st Department of Medicine, Institute for Internal Medicine, Medical and Health Science Center, University of Debrecen
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1. INTRODUCTION

Electroencephalography is an indispensable tool in both research and clinical practice, because the electromagnetic oscillations of the brain cannot be detected by any other investigating methods, except for magnetoencephalography. In addition, EEG has excellent temporal resolution in millisecond scale.
EEG is of great importance in epilepsy, because epileptic dysfunction is characterized by electromagnetic oscillations. Other imaging methods demonstrate only the consequences of the abnormal electrical functioning of the brain: changes in blood flow or biochemical, metabolic alterations. Conventional visual EEG analysis is not eligible to assess those subtle details of the electrical activity of the brain, which might be relevant in research or clinical practice. Computerized EEG analysis might be the solution for this problem. In our studies two techniques were used: conventional spectral frequency analysis and a new technique called LORETA (Low Resolution Electromagnetic Tomography), which localizes the sources of EEG activity in 3-dimensional space.

2. OBJECTIVES

Sources of resting-waking EEG activity were analysed in different groups of epilepsy patients using LORETA. Three, epilepsy-related issues were addressed.

1. Epilepsy is the result of interactions among the acquired and inherited factors. Acquired causes can be investigated with advanced neuroimaging methods, but an appropriate method to evaluate genetically determined epileptic liability is still lacking.
We investigated the "pure epileptic predisposition" (increased seizure propensity without epilepsy) in otherwise healthy, non-medicated persons who presented with one or a few generalized tonic-clonic seizures (GTCSs) precipitated by natural, near-physiological events, but never displayed spontaneous (non-provoked) seizures.

2. Prior quantitative EEG (QEEG) studies disclosed increased theta (4-7 Hz) power all over the scalp in partial epilepsy patients (PE). One would expect that theta activity is increased (according to the conventional EEG interpretation) nearby the epileptogenic lesion or epileptogenic area. Because of methodological limitations the prior studies could not

confidently clarify this contradiction. **The issue of diffuse theta activity in PE was re-investigated and cortical sources of theta activity were localized using LORETA.**

3. Interictal epileptiform discharges (IEDs) are not always clinically silent because IED-related derangement of cortical functions might cause clinical symptoms in sensory, motor and cognitive domains, strictly time-locked to the IEDs. However, impairment of cognitive functions can outlast the duration of the spike-and-slow wave complexes. Explanation of this phenomenon is still lacking. Based on the overall behavior of neuronal oscillations we assumed that focal spike-and-slow wave complexes - mainly the wave component of them – behave as a decreasing oscillation and might contribute to the electrophysiological delayed effect. **We investigated the time course and spatial distribution of IED- related cortical dysfunction.**

3. PATIENTS AND METHODS

3.1. Patients

3.1.1. Quantitative EEG abnormalities in „pure" epileptic predisposition

Patients between age of 10 and 30 years with normal medical history were prospectively sorted out of those who displayed one or more generalized tonic-clonic seizures (GTCS) precipitated by 24 or more hours of sleep deprivation but never had unprovoked seizures. The patients who were diagnosed as having "sleep deprivation seizures" did not receive medication and were followed at regular visits for at least two years to exclude beginning epilepsy. At the end of the two-year period 10 eligible patients remained in the study. An age- and sex-matched control group composed of 10 healthy persons was selected from our normative EEG database.

3.1.2. Sources of theta activity in partial epilepsy patients

Group 1 was recruited from newly diagnosed, untreated PE patients (5 males, 4 females; age: 13-43 years; average age: 22.6 years). Standard neurological investigation, EEG and MRI (carried out according to the epilepsy protocol, at 1.5 T magnetic field strength) was performed in these patients as parts of the routine epilepsy evaluation protocol.

Group 2 was recruited from chronic, already treated PE patients (8 males, 23 females; age:13-56 years; average age: 27 years) who already fulfilled the same evaluation protocol.
As to investigate the potential effect of the MRI-defined lesion on theta activity three new groups were created according to the side of the lesion from patients of Group 1 and Group 2. 20 patients in the nonlesional group, 10 patients in the left hemisphere lesion group and 8 patients in the right hemisphere lesion group were compared. Two patients with multiple cerebral lesions escaped this analysis.

3.1.3. Focal interictal epileptiform discharge-related cortical dysfunction

In our retrospective study the EEG data of non-lesional focal epileptic children (aged between 6 and 14 years) were analysed, who did not receive antiepileptic medication at the time of EEG evaluation and had a single spike focus in the EEG record. 8 children with idiopathic partial epilepsy with rolandic spikes (BERS) and 3 children with cryptogenic epilepsy were enrolled (8 males, 3 females; age: 6-14 years; average age: 8.45 years).

3.2. Methods

3.2.1. Resting-EEG registration and quantitative EEG analyses

All EEGs were recorded with the same sort of digital EEG equipment (Micromed BQ 3200). EEG was recorded from 19 active electrodes plus earlobe referential electrodes according to the general technical and quality criteria for quantitative EEG analysis. 30-40 minutes EEG was recorded in relaxed-waking, eyes-closed state of the patients. 2-second artefact-free epochs of spontaneous, waking activity characterized by continuous alpha rhythm with posterior voltage maximum were selected for quantitative analysis.

Fast Fourier transform (FFT) of the selected samples was carried out from 0.5 Hz to 40.0 Hz, frequency resolution was 0.5 Hz. Spectral analysis was carried out by means of the NeuroGuide 2.5.6. software. Absolute power was computed for each frequency point (spectral power, microvolts^2/Hz). The data were compressed into conventional broad frequency bands (delta: 0.5-4 Hz, theta: 4.5-8 Hz, alpha:8.5-12 Hz, beta:12.5-40 Hz) and into very narrow bands (VNB, 1 Hz resolution). Also age-adjusted, Z-transformed absolute power values were computed using the NeuroGuide normative database. Z transformation permitted the

evaluation of the deviation of the individual spectral data from the theoretical mean (Z = 0) of the healthy population of the same age.

LORETA was used to localize the cortical sources of EEG activity and to compute the current source density of the sources. Source localization is based on the solution of the electromagnetic inverse problem and computes the cortical localization of the sources underlying EEG activity from the scalp distribution of the electric field. LORETA method assumes that neighbouring neurons are simultaneously and synchronously activated. LORETA subdivides the grey matter compartment in 2394 voxels with a spatial resolution of 7 mm and computes current source density (Ampers/meters squared) for each voxel, this is called „activity". Raw LORETA values underwent age-adjustment and Z-transformation using the LORETA Normative EEG Database (LORETA-Z).

3.2.2. Quantitative EEG abnormalities in „pure" epileptic predisposition

60 epochs were averaged in each patient and control person. Detailed spectral analysis was not carried out in this study. Individual LORETA analysis was carried out in each patient and control person, and raw LORETA values were averaged in patient and control groups. VNB values were compressed into four frequency bands (delta, theta, alpha, beta). The patients and the controls were compared by independent t-tests. T-values corresponding to $p<0.01$ were labelled as statistically significant.

3.2.3. Sources of theta activity in partial epilepsy patients

60 epochs were averaged and Z-scored power spectrum was computed for 19 derivations in each patient. Individual spectral data were averaged for Group 1 and Group 2.
Spectral analysis was focused on the theta band (5.0-8.0 Hz) but also the neighbouring bands: delta (1.0-4.0 Hz) and alpha (9.0-12.0 Hz) were considered.

Each individual LORETA analysis was performed at the frequency of the maximum positive Z-scored theta value within the 5.0-8.0 Hz frequency range as established in the individual EEG spectrum. Individual LORETA values were averaged for LORETA group analysis.
To evaluate the potential effect of the MRI-defined lesion on ipsilateral and contralateral theta activity maximal individual Z-scores in both hemispheres were averaged in three groups (left

hemisphere lesion, right hemisphere lesion and nonlesional group) and statistical analysis was carried out.

3.2.4. Focal interictal epileptiform discharge –related cortical dysfunction

Individual analysis was carried out in each patient. 20-30 epochs of EEG activity each containing a single spike-and-slow complex was selected (Spike epoch). Thereafter, 2-second epochs immediately following the Spike epochs (S) were selected (PostSpike-1 epochs, PS1), followed by the selection of PostSpike-2 (PS2) and PostSpike-3 (PS3) epochs each of 2 seconds. Finally, 2-second epochs that were located at least 10 seconds from the last Spike epoch (InterSpike epochs, IS) were selected.

The frequency of the slow wave component of the focal spike-and-slow complex was determined from the Z-scored power spectrum. Very narrow band LORETA-Z analysis was carried out at this frequency. Picture-series were created for each patient in order to assess the anatomical extension and degree of maximum LORETA-activity across the averaged S, PS1, PS2, PS3 and IS epochs.

4. RESULTS

4.1. Quantitative EEG abnormalities in „pure" epileptic predisposition

Statistically significant ($p<0.01$) decrease of alpha activity was found in the persons with epileptic disposition (as compared to the controls) in the medial and lateral parts of the cortex above the level of basal ganglia. These alpha differences were distributed asymmetrically at the cortical convexity, being more widespread in the right hemisphere than in the left. Maximal alpha difference was found in the left precuneus. The only statistically significant beta difference was localized to the left precuneus, too.

No statistically significant ($p=0.3$) difference emerged between the two groups in the delta and theta bands. An overall tendency for bilaterally increased delta and theta activity was found in the persons with epileptic predisposition as compared to the controls. Greatest delta differences were found bilaterally in the medial and basal prefrontal cortex. Greatest theta

differences were found in about the same parts of the frontal cortex and in the nearby basal temporal cortex.

4.2. Sources of theta activity in partial epilepsy patients

Spectral findings

The theta peak was separable from the delta and alpha peak values in the individual Z-spectra. The theta spectrum showed simultaneous increase, peak, and decrease of power in most or all derivations in the majority of the patients, suggesting a topographically diffuse process that differs from those seen in the delta and alpha bands.

Each patient in Group1 showed Z-scored power within the ± 1 Z range. Remarkably, the scalp-averages (the average of Z-scored theta power values across the 19 derivations) were scattered around zero.

On the contrary, all Z-scored theta values in Group2 were positive, and the mean values differed considerably from Z=0. There was a stepwise increase of the Z-scores from 1 Hz to 7 Hz as demonstrated by the scalp-averages and the increasing number of the Z>1 values in the individual derivations. All but one values returned below the Z=1 level at 9 Hz and the faster frequencies in the alpha range.

LORETA group analyses

In Group 1 contiguous areas consisting voxels of increased (Z>0) or decreased (Z<0) activity were found in both hemispheres. Three areas of increased theta activity were labelled as the parietal theta area (PTA)**,** frontal theta area (FTA), and temporal theta area (TTA). The theta areas were roughly symmetrical and were separated by areas of lesser activity.

Maximum activity in the left and right PTA was consistently found in the superior parietal lobule. In the left and right FTA maximum activity was confined to the superior and medial frontal gyri and a limited part of the cingulate gyrus. Maximum abnormality in the TTA was located in the fusiform gyri bilaterally.

In Group2 the amount of theta activity showed an overall increase across the entire cortex, but the TTA, PTA, and FTA were also identifiable. The degree of the maximum theta abnormalities at 7 Hz was about two to four times greater in Group2 than in Group1. In addition, in the PTA and FTA the center of the abnormality shifted toward the midline cortex (precuneus and cingulate gyrus) as compared to the loci of the maximum abnormalities in Group1.

LORETA individual analyses

LORETA individual analysis was carried out in each patient. We found that one of the three theta areas was preferentially activated (that is, showed greater Z-values than the remaining theta areas) in all but three patients. Maximum theta values were found at 6 or 7 Hz in most patients.

Effect of the MRI lesion on theta activity

No statistically significant differences emerged between the three groups ($p=0.38$) suggesting that the presence and laterality of the lesion did not significantly influence the emergence and degree of the theta abnormality.
Our findings suggest that preferential activation emerges in the theta center nearby the epileptogenic process but further investigations are necessary to confirm this association.

4.3. Focal interictal epileptiform discharge –related cortical dysfunction

The spatial extension of the abnormality was the greatest in the Spike epochs and showed an overall tendency for gradual spatial shrinking in the PS1, PS2, PS3 and IS epochs However, the individual time course of the decrease of the abnormality differed from patient to patient. As to show the average tendency the data of the patients were averaged. Paired t-tests showed statistically considerable difference between the Spike and PS1 epochs ($p<0.0001$), between PS1 and PS2 ($p=0.004$) but not between PS2 and PS3 ($p=0.276$) and PS3 and IS epochs ($p=0.070$).

5. DISCUSSION

5.1. Quantitative EEG abnormalities in „pure" epileptic predisposition

We were the first to investigate pure epileptic predisposition without epilepsy. Our results did not confirmed the hypothesis that epileptic predisposition is characterized by increased neuronal synchronisation. However, the topography of increased delta and theta activity in these persons and in patients with idiopathic generalized epilepsy was similar, the degree of the abnormality was rather dissimilar. Nevertheless the patient group and control group are similar in clinical-EEG aspects: spontaneous seizures are lacking in both groups and the differences between patients and controls are not statistically significant.

Epileptic predisposition was characterized by statistically significant decrease of alpha activity in most of the cortical mantle, and a statistically significant decrease of beta activity in a circumscribed part of the left parietal cortex, as compared to the control group. The left precuneus was the site of the maximal alpha and beta differences.

The neurophysiological interpretation of decreased alpha and beta activity in the context of seizure liability is not easy. As far as is known, the relationship between alpha variables and seizure propensity has never been investigated. Beta decrease is usually interpreted as a non-specific sign of impaired cortical function. Beta power was proposed as a biological marker of GABA-mediated anticonvulsive drug effects thus indirectly suggesting that beta decrease might be associated with increased seizure propensity. However, also opposite results were published and, in any case, drug-modified cortical GABA-ergic function is only one component of seizure liability.

Similarly, the significance of maximal alpha and beta decrease in the left precuneus remains uncertain. Concordantly with this finding, interictal dysfunction of the precuneus and posterior cingulate cortex in patients with generalized seizures was demonstrated in a functional MRI study. The strategic role of this area in regulating physiological arousal processes and perhaps also epileptic activity argues for further, targeted investigations.

5.2. Sources of theta activity in partial epilepsy patients

Authors who investigated the broad-band power in partial epilepsy patients did not emphasize the problem that the theta band comprises an uncertain number of rhythmic oscillations. Using VNB analysis we found oscillations confined to a single VNB thus confirming the prior recommendation for narrow band analysis.
Prior quantitative EEG studies did not investigate drug-treated and untreated PE patients separately, although the effect of antiepileptic drugs on EEG activity was already known. **This was the first quantitative EEG study carried out in a group of PE patients with beginning, untreated epilepsy.** Albeit this group was small, analysis of the spectral results disclosed that the overall increase of theta activity is not an immanent characteristics of PE. This finding was greatly refined by source analysis. LORETA demonstrated that not the entire cortex but three, anatomically distinct areas in each hemisphere showed increased theta activity. Importantly, these theta maxima are topographically not identical with those of healthy persons. The explanation of this phenomenon is not known, the spatial relationship between the epileptogenic lesion and the activated theta area might encourage further investigations. A recent study also supports our hypothesis: more or less rhythmic interictal theta activity in the frontal derivations was frequently found in patients with frontal epilepsy but rarely in temporal lobe epilepsy patients.

The frequency-dependence of activity was observed in most individual spectra and LORETA analyses suggesting that, defining sharp borders between neighbouring frequency bands poorly reflects physiological reality. The individual dispersion of the VNBs with maximum activity indicates biological variability concerning the working frequency of the theta network. The individual peak frequencies should be emphasized in planning targeted studies and in the evaluation of individual persons.

Our spectral results confirmed the presence of diffuse theta increase in patients with chronic, treated PE as described in prior studies. Areas of increased theta activity were identifiable in this group, too. To identify the reason for this theta increase in the treated group was not purpose of our study; antiepileptic medication, particularly carbamazepine, and cumulating changes in the course of the illness, e.g. neocortical volume loss in T1 weighted MRI images might be contributing factors.

5.3. Focal interictal epileptiform discharge –related cortical dysfunction

Up to now, a single experimental study described the delayed EEG effect of epileptiform transients beyond the duration of the IED: the decrease of axonal firing of hippocampal neurons outlasts the IED for about 2 seconds but even for longer periods after clusters of spikes. **We were the first to verify that the delayed EEG effect also exists in large neuronal populations. Our findings confirmed** the hypothesis that the IED-related cortical dysfunction behaves as a decreasing oscillation that lasts for a few seconds beyond the duration of the IEDs, and the delayed effect can quantitatively be assessed in space and time. Its neuronal basis is not known but several mechanisms exist that can generate and sustain rhythmic phenomena in epileptically functioning neuronal ensembles. Even the InterSpike epochs - far from the IEDs - show some degree of focal abnormality, as compared to the rest of the cortical mantle. This is in accord with a recent intracranial EEG study demonstrating that locally increased synchronization in the interictal state is characteristic to the epileptic cortex in focal epilepsy.

Neurophysiological testing parallel with EEG was not performed thus it is not known to which degree the delayed effect contributes to cognitive impairment symptoms. Only the anatomical distribution at the frequency of the wave was investigated in this study. However, the complex, mutual interdependence of the physiological oscillations at diverse frequencies in the brain implies that IEDs may cause interference with some other oscillations underlying cognition and other cortical activities.

6. SUMMARY

The epileptic cortex is characterized by altered, mainly increased neuronal synchronization, which exists also in interictal state. An EEG-source localization method LORETA (Low Resolution Electromagnetic Tomography) demonstrates the synchronously activated neuronal populations in the 3-dimensional space. For this reason it is an appropriate method to localize the sources of abnormal cortical activity in epilepsy patients. Three, epilepsy-related issues were investigated using LORETA, the main findings are summarized here.

1. The first study was designed to analyse EEG-LORETA data of ”pure” epileptic predisposition (without epilepsy) in persons who display generalized tonic-clonic seizures

precipitated by natural, near-physiological events, but never display spontaneous seizures. Increased degree of neuronal synchronization was assumed based on findings of a few animal experiments. Our findings suggested similar dysfunction in some cortical areas of the patients but the results did not survive statistical analysis. On the other hand, impaired cortical function was found in various cortical regions in patients with pure epileptic predisposition. In the future, targeted studies should be planned to investigate these cortical regions as to understand the mechanisms of seizure susceptibility in the human being.

2. In our second study we demonstrated that the enigmatic, diffuse EEG theta activity that was described in untreated partial epilepsy patients is not diffuse at the level of the cortical generators. Instead, it reflects the increased activity of three, anatomically distinct cortical areas (theta areas). Furthermore, our findings suggest (but do not prove) that the localization of the epileptogenic lesion might increase the degree of theta-synchronization bilaterally, particularly in the nearby theta area. Our investigations permit comprehensive evaluation of the role of theta-generators in epileptic condition.

3. It is theoretically possible that abnormal oscillations that survive the interictal epileptiform discharge (IED) might contribute to IED-related transient neurological and cognitive deficit symptoms lasting beyond the duration of the IEDs. In this study we were the first to verify the ”delayed effect” of IEDs in large neuronal populations, and we developed a method to quantitatively assess and localize IED-related dysfunctions.

Overall, we testified that LORETA is an appropriate method to investigate issues of neurophysiological and clinical importance with high accuracy. Our findings are of methodological and heuristic importance concerning forthcoming investigations in the field of epilepsy.

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1. **Puskás, S.**, Bessenyei, M., Fekete, I., Hollódy, K., Clemens, B.: Quantitative EEG abnormalities in persons with "pure" epileptic predisposition without epilepsy: A low resolution electromagnetic tomography (LORETA) study.
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