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Treatment of epilepsy and brain networks

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1. INTRODUCTION

Epilepsy, in the narrowest sense, means the occurrence of spontaneous epileptic seizures. The prevalence of epilepsy is 0.5-1.0 percent in the whole population, but the extension of the definition to “seizure disorders” indicates an even higher rate. Epilepsy impairs quality of life in all ages and all other (biological, psychological, sociological) dimensions, and it constitutes a significant percentage of medical expenses. All this demonstrates and explains the relevance of experimental and clinical epilepsy research. Unfortunately, the traditional approach to epilepsy (which means knowing the disease's neurophysiological basis and the treatment strategies based on this knowledge) restricts research and clinical application potentials. As for the medical treatment, no preventive or causal therapy exists, and the symptomatic (seizure preventing) treatment results in seizure freedom in only 65-70 percent of the patients. It has become apparent that improvement is hardly possible without a broad-ranging reconsideration of present neurophysiological and pharmacological knowledge and a designation of new research directions. The application of the so-called "network concept" is a new approach in epilepsy research. It is not an epilepsy-specific conception but an extensive theory adopting systems biology principles for neurological diseases, including epilepsy. According to this theory, brain functions and executions do not emerge in brain centers but networks of different scales (size, complexity). Events recorded at small-scale size are not necessarily valid on the level of larger-scale size and complexity. This dissertation summarizes the traditional and "network" fundamentals first, then the application of the "network concept" is presented to the medical treatment of epilepsies.

2. OBJECTIVES

2.1. The aim of the dissertation I. (in focal epilepsy)

In a retrospective study, we analyzed how clinical-biological factors act upon the dynamics of focal epilepsy (FE) patients' large-scale interictal brain network, which on this scale is called **"connectivity".** We highlighted the network changes related to medical treatment, but our investigation also extended to other factors' effects to get a complex picture about which factors determine the connectivity parameters. We hypothesized that if all brain states result from network activity, then there is a difference in the network's status in the treated and the untreated conditions.

2.2. The aim of the dissertation II. (in generalized epilepsy)

In a prospective, self-controlled study, we analyzed the medium-scale brain network dynamics in idiopathic generalized epilepsy (IGE) patients, which on this scale is called **"synchronization"**. According to our hypothesis, a successful treatment (responder patient and seizure-free state) reduces EEG synchronization in the 0.5-8.0 Hz frequency range, while an unsuccessful treatment (non-responder patient, persisting seizures) equals persisting EEG-abnormality. In other words, we assumed that a drug-induced decrease of synchronization is the predictive biomarker of successful treatment.

3. PATIENTS

University of Debrecen Clinical Center Divison of Neurology has outpatient care for epilepsy patients since 1981. The patients' structured data is collected in a self-developed database (Epi-Stat), including the patients' clinical, EEG, and neuroimaging results. This database enables us to carry out prospective and retrospective studies.

3.1. The retrospective study of focal epilepsy patients' brain network and the modifying factors affecting it

We enrolled 232 cryptogenic and symptomatic FE patients who were older than four years of age, registered between 2008 and 2016, had no comorbidity or other circumstances that could have affected the study, and whose EEG recordings were suitable for analysis. The healthy control group consisted of 77 patients. Based on neurophysiological considerations, we defined six factors. In our opinion, these factors can modify the connectivity parameters:

- **etiology:** cryptogenic or symptomatic
- **family:** seizure disorder in the family – positive or negative
- **onset:** according to age groups: 4-10 years, 11-20 years, 21-93 years
- **seizure type:** only partial seizures (PS), partial and secondary generalized seizure (PSSG), only secondary generalized seizures (SG)
- **duration:** number of years between the first seizure and the analyzed EEG recording. Groups: < 1 year, 1-10 years, 11-20 years, >20 years
- **treatment:** treated or untreated

3.2. Investigation of the response of idiopathic generalized epilepsy patients to treatment

Patients with untreated idiopathic childhood and juvenile absence epilepsy (AE) or juvenile myoclonic epilepsy (JME) were enrolled in the investigation. Besides the diagnosis, the inclusion criteria were

- untreated state,
- a good quality EEG record suitable for analysis, and
- lack of any condition that could affect brain function.

We included 40 patients, but only 31 patients' data could be analyzed. Each patient had two EEGs: the first one (EEG1) in the untreated condition, and the second one (EEG2) 6 months after the first visit when the therapeutic effect or the lack of it could be determined. Then we classified the patients into a seizure-free (responder) group or a persisting seizure (non-responder) group.

4. METHODS

4.1. The EEG record and epoch selection

We recorded EEGs and selected EEG epochs for analysis with the same method in both studies, including patients and healthy control persons.

EEGs were recorded under standard conditions in the EEG Laboratory by well-trained neurophysiology technicians using Micromed Brain Quick Digital EEG equipment. EEG examinations were carried out in the supine position, eyes-closed, waking-relaxed state for 25-30 minutes. Electrodes were placed according to the international 10-20 system. 21-channel EEGs were recorded from standard scalp sites and the earlobes against Fpz sampling reference; after that, we recomputed EEGs against a mathematically linked reference. Sampling rate was 256/second. We selected 90 two-seconds epochs for quantitative analysis, according to the following criteria:

1. presence of continuous physiological, waking alpha activity, with voltage maximum in posterior regions,
2. absence of epileptiform potentials and other nonstationary elements or artifacts,
3. absence of patterns indicating drowsiness or arousal.

NeuroGuide Version 2.8 software was used for this procedure.

4.2. Low Resolution Electromagnetic Tomography (LORETA).

The quantitative EEG analysis in generalized epilepsies was focused on synchronization and was carried out by LORETA software. This method uses voltage values of the potential field measured on the scalp, and by means of neuroanatomical-neurophysiological knowledge and a mathematical constraint ("smoothness assumption"), it determines the magnitude of transmembrane currents produced by cortical EEG generators ("LORETA activity", current source density, CSD, Ampers/m^2) in 2394 cortical voxels. Voxel-wise CSD values appear on a standard brain template in a color-coded way. The LORETA method can be used at any frequency range allowed by the sampling frequency. Data can be saved in Excel tables and are suitable for further processing.

In generalized epilepsy patients, we calculated an average LORETA activity in each EEG sample, including all voxels in the 0.5-8.0 Hz frequency range. This way, a single value represented the synchronization in the untreated condition, and another value indicated the synchronization in the treated condition.

4.3. LORETA Source Correlation (LSC)

The LORETA Source Correlation (LSC) method, which applies LORETA values, was used to investigate the network in focal epilepsy. The software computes CSD values for larger cortical areas (ROI, region of interest) by averaging the voxel-wise values. One ROI corresponds to one larger or 2-3 smaller cortical gyri. We calculated CSD values for very narrow bands (VNB) of 1 Hz bandwidth from 1 to 25 Hz in 23 ROIs in each hemisphere. This procedure resulted in time series for all VNBs and all ROIs. The next step of this analysis was the Pearson correlation coefficient ("r") calculation between all pairs of ROIs in one hemisphere for each of the 25 VNBs, which resulted in [(23 x 23) -1] x 25 "r" values for one hemisphere. The values indicate intrahemispheric cortico-cortical functional connectivity (EEGfC), which were adjusted for age and Z-transformed for further computations.

5. STATISTICAL ANALYSES

5.1. In the analysis of connectivity in focal epilepsies, an exploratory comparison was performed between the total patient and control groups. Five hundred six topographical connections in each hemisphere for the 25 frequency bands meant a total of 25300 Student t-tests for both hemispheres. Statistically significant ($p<0,05$) differences were highlighted by FDR (false discovery rate) correction.

Factor analysis was based on analysis of variance (ANOVA) and post hoc Tukey tests. By using the obtained data, we created 15 SPNs (statistical parametric network). FDR-based thresholding emphasized the statistically significant ($p<0,05$) differences.

5.2. In generalized epilepsies, we searched for a correlation between treatment results (responder vs. non-responder state) and synchronization changes based on averaged LORETA activities (reduced vs. not reduced). We analyzed the results of the contingency table by Fisher's exact test.

6. RESULTS

6.1. The investigation of focal epilepsy patients' brain network and the factors affecting it

6.1.1. The comparison of the total FE group and the NC group

Increased EEGfC was found in the FE group in the 3-7 Hz range with a maximum at 6 Hz. Abnormal connections emerged predominantly between the frontal, temporal, and lateral parietal ROIs. Decreased connectivity was detected at 11 Hz in the FE group between more posterior ROIs as compared with the NC group (cuneus, the three lateral temporal, and the lateral parietal ROIs).

6.1.2. The results of factor analysis

Four factors (etiology, seizure type, duration, treatment) had independent and statistically significant effects on the connectivity parameters. Two factors (family and onset of the disease) had no statistically demonstrable influence on the network.

6.1.2.1. Etiology

In symptomatic FE patients, reduced connectivity was found compared to the cryptogenic FE group at 11-13 Hz between the frontal and parietal ROIs in both hemispheres. In symptomatic FE patients, reduced connectivity emerged at 15 Hz between the temporal and parietal ROIs.

6.1.2.2. Seizure type

In the SG group, increased connectivity was demonstrated compared to the PS and PSSG groups at 10 and 11 Hz bilaterally, predominantly in the left hemisphere, in the frontal, central temporal, and frontal-parietal areas. The difference between the SG and PSSG groups was almost identical at 10-11 Hz.

6.1.2.3. Duration of the disease

We found a statistically significant difference at 6 and 7 Hz during the group comparison. Increased connectivity was associated with a longer duration of the disease in each comparison. The biggest difference emerged between the groups with the shortest and the longest duration of the disease in the frontal central and parietal connections. There was a linear relationship between the increase of connectivity and the disease's duration, except for the occipital region, where there was no difference between patient groups. We found decreased connectivity between 11-18 Hz, which was also the most prominent between the groups with the shortest and longest disease duration.

6.1.2.4. Therapy

The untreated group showed decreased connectivity as compared to the treated group at 5-7 Hz. The most prominent topographical difference appeared at 6 Hz and included all the connections. Increased connectivity was observed in the untreated group compared to the treated group at 11 Hz in the left hemisphere and 20 Hz bilaterally involving most of the connections.

6.2. Investigation the response of IGE patients to treatment

Medical treatment resulted seizure-free state in 26 patients (responders). Five patients (3 AE, 2 JME) were non-responders. All but one (patient No. 42) responder patients' average CSD value were higher in the treated than in the untreated state. By contrast, all five non-responders' average CSD values were higher in the treated than in the untreated state. A statistically significant relationship emerged between the favorable outcome and CSD reduction and between the unfavorable outcome and an increase of CSD.

7. DISCUSSION

7.1. Connectivity and the factors affecting it in the FE group

7.1.1. FE-NC global connectivity differences

Our work is the first study in which EEG functional connectivity was analyzed from a topographical point of view and in narrow frequency bands. This, compared with the traditional broad band analysis, presents more realistic results because the natural oscillations of the brain occur at 1-2 Hz bandwidth. In our investigation, we demonstrated patient-control differences, which affected one or two VNBs selectively. With this finding, we proved that the theory mentioned above is valid in human EEG-based connectivity.

According to the present concept, the network of FE patients, a heterogeneous group in every aspect, shows no common pattern because it develops in each patient individually. In contrast, we could discover common patterns in the investigated patient group's network, despite the group's significant variability in every aspect.

Increased connectivity in the theta band (particularly at 6 Hz) was characteristic in the FE group; however, it was also demonstrated in the network analysis of 259 neurological patients (epilepsy and non-epilepsy patients). The 6 Hz frequency is the thalamo-cortical rhythm; however, this is also the limbic system's prominent rhythm. It harmonizes the two systems' activity, so it is not surprising that an increase can be observed in this activity in different brain disorders.

Another feature across patient groups is the reduction of alpha connectivity between the posterior ROIs. Our analysis showed reduced internal connectivity of this region in the FE group, and the same result was found in the already mentioned 259 neurological patients. These results correspond to a high-level and not an etiology-specific vulnerability of a highly organized cortical coordinative area (the so-called" rich club region").

7.1.2. The clinical conditions that affect the network in FE

Factors affecting the brain network of FE patients have not yet been investigated. Four out of the six investigated factors (etiology, seizure type, duration, treatment) had an independent modifying effect on connectivity. Two of them (family and onset) did not have a statistically demonstrable influence.

The effect of diverse biological factors appeared in a few highlighted frequencies. Four factors (etiology, seizure type, duration, treatment) affected the so-called upper alpha band, two (duration and treatment) the upper theta (6-7 Hz) range. The results suggest that the network-modifying factors mainly affect the theta and alpha frequencies involved in brain integration.

7.1.2.1. Etiology

We found reduced connectivity in the upper alpha band in the symptomatic FE patient group compared to the cryptogenic FE group. Alpha is an integrative rhythm present in large areas of the brain. For this reason, it is plausible that in the symptomatic FE group (which is characterized by evident brain structure damage), the decrease of alpha-connectivity is higher than without a detectable brain pathology, that is, in the cryptogenic FE group. It is well-known that most brain diseases reduce the synchronization of alpha activity. Our work suggests that on the network level, alpha-connectivity decreases as well.

7.1.2.2. Onset of the disease

Epileptogenesis is a long process during which the normal brain network transforms into an epileptic network. We assumed that an insult affecting the developing brain causes more serious network damage than an effect harming the brain at an older age. Our results did not support this concept. It is necessary to mention that we defined the time of the first non-provoked seizure as the onset of the disease. In reality, epileptogenesis and the network's transformation start long before the first seizure appears, and the onset of the process is known only in a few cases.

7.1.2.3. Seizure type

A cortico-cortical spread characterizes the initial phase of focal epileptic seizures, then a further spread may follow to remote regions, and finally, occasionally, a so-called (secondary) generalization can evolve. We postulated that the degree of cortico-cortical connectivity is lower in those patients whose seizures are localized to a small cortical area without generalization, compared to those whose seizures occasionally, or always, generalize. Our study proved the relationship between increased connectivity measured at 10-11 Hz and the propensity for generalization. From a neurophysiological point of view, it seems evident that the increased cortico-cortical connectivity would subserve the spreading of a focal seizure between the brain areas and generalization.

7.1.2.4. Duration of epilepsy

Permanent epileptic dysfunction causes cumulative damage in the brain's structure and function, proportional to the duration of dysfunction. On the network-level, we confirmed a relationship between the disease's duration and the diffusely increased connectivity (sparing the occipital area) at 6-7 Hz and between the disease's duration and the reduced connectivity at 12 Hz.

7.1.2.5. Treatment

Our results confirmed the hypothesis that there is a difference between the treated and untreated condition's connectivity in focal epilepsy. It is necessary to consider that we did not compare the same patient's untreated and treated condition during the evaluation. Nevertheless, the treated cohort can be considered successfully treated on a group level because the vast majority of the patients were seizure-free or had only 1-2 seizures per year. The treatment proved to be an independent factor that influences connectivity. There is no satisfactory explanation for the increased connectivity (at 5-7 Hz) in the treated condition. The decrease of connectivity appears (at 11 Hz and 20 Hz) at the motor cortex's frequencies. It can be interpreted as the loosening of the relationship between the motor cortex and other cortical regions, consistent with antiepileptic compounds' inhibitory effects on the extension of seizure activity. In clinical practice, reducing the number of SG seizures is regarded as a success, even if the focal seizures or auras that do not evolve into more severe seizures persist.

7.2. Evaluation of the response of AE and JME patients to treatment

Our findings confirm the hypothesis that there is a relationship between the responder state and the reduction of 0.5-8.0 Hz global EEG synchronization and between the refractor state and the persistence of global EEG synchronization in generalized epilepsies. Therefore, this effect can be evaluated as a predictive, therapeutic marker. The results are also related to personalized medicine and predictive, therapeutic biomarkers, for which there is a growing interest in the 21st century. In our work, the therapeutic effect's prediction applies only for a short period of time, but it may be extended for a longer period, according to the literature. These results suggest that our method may be successful in IGE syndrome with GTCSs only and focal or non-categorized epilepsies, but further investigations are needed to confirm this theory. Predictive data are essential in patients with rare (1-3 seizures/year) but severe seizures when months or years might go by before an appropriate medication is found.

Another important conclusion of our investigation is that the parallel clinical-EEG change was independent of the antiepileptic drug type and the molecular-subcellular mechanisms of action. It corresponds to the principle of pharmaco-EEG (according to which the clinical improvement goes parallel with the reduction of the EEG abnormality) and the adaptation of the systems biology's thesis to the nervous system's pharmacology. The latter means that the effect on the higher organized levels (brain networks, synchronization) cannot be derived from the effect on a lower level (molecular mechanism of action). Results already exist in experimental pharmacology proving this principle, but we were the first to prove it in humans.

8. SUMMARY

With respect and appreciation for prior epilepsy research results, it can also be concluded that, unfortunately, we are "going around in circles" regarding neurophysiological principles and medical treatment. In particular, medical treatment indicators do not improve to such an extent that we hoped for despite drug development expenses. The demand of our time, the personalized treatment can only be accomplished to a limited extent because unpredictable uncertainty may occur in each case regarding therapeutic effects and side effects. It seems that focusing on isolated details may cause the failure. A new approach regarding brain and epilepsy is the "network perspective", which emerges from the combined application of the brain networks' science and the systems biology principles on the central nervous system. Only a few investigations exist in epilepsy following this approach; however, the results so far suggest that the network perspective is the tool that enables advances in those fields where traditional methods fail. The network perspective's essence is that the events occurring at a small, medium, and large brain scale are not analyzed individually but assessed as a whole (network), including the network elements (cortical regions) and their anatomical connections. Complex mathematical methods can examine global functioning. In this dissertation, we investigated EEG-based network functions and indicators.

Our study conducted on patients with focal epilepsy revealed large-scale interictal network features and the factors that influence them. Most of our results have neurophysiological-clinical interpretations as well as heuristic value for potential future investigations.

In generalized epilepsy, we found a connection between the clinical outcome (seizure control) and a quantitative EEG-network indicator. The relationship can be interpreted as a therapeutic, predictive biomarker and applied in practice.

We do not believe that the network research would contradict previous results and render investigation methods used before the network-era. Various research strategies and solutions, the synthesis of old and new results is the way we should follow to understand and treat the disease.

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List of publications related to the dissertation

1. Clemens, B., **Dömötör, J.**, Emri, M., Puskás, S., Fekete, I.: Inter-ictal network of focal epilepsy and effects of clinical factors on network activity.
   Clin. Neurophysiol. 130 (2), 251-258, 2019.
   DOI: http://dx.doi.org/10.1016/j.clinph.2018.11.022
   IF: 3.214
2. **Dömötör, J.**, Clemens, B., Puskás, S., Fekete, I.: Decrease of global current source density predicts successful treatment in absence and juvenile myoclonic epilepsies.
   Epilepsy Res. 133, 1-5, 2017.
   DOI: http://dx.doi.org/10.1016/j.eplepsyres.2017.03.006
   IF: 2.491

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