Electrophysiological studies in schizophrenia - gamma activity and microstate segmentation with high density EEG system

Doctoral theses

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INTRODUCTION

Several structural and functional neural network abnormalities have been described in schizophrenia, indicating the nature of the disease, which entails a comprehensive, multi-segmental deficit in the brain involving neural network connections. The disorder is epidemiologically significant, its world-wide prevalence is approximately 1%. The diagnosis of the disorderis based on ICD-10 (International Classification of Disease) and DSM 5 (Diagnosis and Statistical Manual of Mental Disorders) criteria.

The etiology of schizophrenia involves several factors, including potential genetic, psychosocial and neurodevelopmental causes. In terms of neurobiological backgrounds, various neurochemical, structural and genetic alterations may play role in the emergence of the disorder, but current pathophysiological theories and neurobiological abnormalities are only partially able to explain the symptom. Thus, a widely accepted theory explaining the disease comprehensively, both its course and its diverse symptoms, has not yet emerged.

Gamma band oscillation (GBO) can be investigated by non-invasive EEG recordings in various task situations or in resting-state. Former studies confirmed that sensory-evoked gamma-

response is reduced in patients with schizophrenia as compared to healthy controls. Furthermore, in cognitive tasks, reduction of gamma-response was found in patients with schizophrenia compared to controls in selective attention and working memory tasks. These task-related gamma disturbances were mostly reported in the lower gamma frequency range - 30-50Hz, especially around 40Hz. While much work has been devoted to investigations with sensory-evoked and cognitive paradigms, resting-state gamma activity has been rarely studied. Recent resting-state studies, using fMRI, showed evidence that task free/resting-state networks correlate with task-positive networks.

In the last 10 years several new analytical methods have been applied to high electrode density EEG recordings. Lehman et al. described first that the spatial configuration of brain electric fields on the scalp remains quasi-stable for a short periods of time and represents “functional microstates” associated with the activity of different intracranial generators that form large scale neuronal networks. These microstates have been considered as building blocks of mentation manifested in EEG. There are four common topographical patterns of microstates, labeled as A, B, C and D. Simultaneous EEG-fMRI has revealed correlations of these microstates with various neural networks, including the auditory (microstate A), visual (microstate B), salience

(microstate C) and fronto-parietal networks (microstate D). An emerging body of evidence suggests that these microstates are temporally distinct electrophysiological components of the default mode network (DMN). Microstates can be characterized by their average duration, occurrence per second and total coverage of time. Furthermore, transition probabilities from one state to another can be quantified. Since data suggest that EEG microstates are associated with large scale brain networks, properties of the microstates can be used to measure large scale brain network operation.

The two studies that are included in the current dissertation looked at resting brain activity from different vantage points. The gamma power spectrum that the first study focused on describes the total power of the gamma oscillations in the examined time window. Since an amplitude increase in the gamma band occurs during the synchronization of cortical networks, and the amplitude increase can be characterized by the frequency power spectrum, gamma power can serve as a measure of the synchronous activity of cortical networks. Deviations in gamma activity can indicate alterations in the synchronous activity of networks, and may affect multiple functions. EEG micro-states have been associated with a number of brain networks. It is likely that the micro-states (scalp potential distributions) are the result of simultaneous

synchronous activity involving multiple networks or network units. Characteristics describing micro-states are metrics of these network structures, and their change suggests impaired system functioning. In summary, both analyzes are used to gain insights into changes in the activity of brain networks.

O**BJECTIVES**

The objective of the first study was to investigate differences in spontaneous gamma activity in patients with schizophrenia and healthy controls (HC). We used high-density EEG to delineate those brain areas in detail where alterations in gamma activity are manifested, and may reflect the operation of networks activated at rest. Focusing on the gamma frequency range of 30-48Hz that was pinpointed by previous studies, we performed a comprehensive, sensor-based analysis of gamma-power differences including the full sensor-space of 256 channels to identify brain areas that may be associated with those networks that contribute to the pathophysiology of schizophrenia. Furthermore, we investigated the association of gamma activity with psychopathological and clinical measures.

The objective of the second study was to build a model that fulfills the requirements of a robust machine learning study. In particular, we used the Support Vector Machine (SVM) to classify patients according to the clinical diagnosis based on electrophysiological data. We used microstate segmentation to extract features from EEG and conducted further analyses to select a subset of these features to feed SVM. We used a 10-fold cross-validation procedure to test the generalizability of the model.

METHODS

We enrolled 106 patients with schizophrenia and 80 healthy controls in the study. Due to technical reasons, such as noisy EEG registration, failed session completion, or ambiguity in the diagnoses we used the EEG data of 70 patients with schizophrenia and 76 healthy controls for the analysis of the two studies.

In the first study sixty patients with schizophrenia (mean age=35.2 years (SD=9.6), male percentage=51.6%) and 76 healthy controls (mean age=32.3 years (SD=10.6), male percentage=35.5%) participated. In the second study 70 patients with schizophrenia (mean age=35.6 years (SD=10.2), male percentage=52.8%) and 75 healthy controls (mean age=32.4 years (SD=10.4), male percentage=35.3%) participated.

The average chlorpromazine equivalent dose was 617.2 mg/day (SD = 333.9 mg). Thirty-three patients were on benzodiazepine medication.

Two minutes of resting-state EEG recordings (Biosemi Active Two system) were obtained while participants sat in a dimly lit room, and were asked to remain still with eyes closed. EEGs were recorded by the 256-channel Biosemi Active Two system (Biosemi Inc., Amsterdam, Netherlands) at a sampling rate of 512Hz, referenced to the vertex.

The recorded data were further processed offline with the Mathworks Matlab „EEGLAB toolbox" and self-made scripts. The statistical analysis was conducted in SAS 9.4 and Matlab.
EEG was re-referenced to the common average potential. A 48-52Hz Parks-McClellan stop-band Notch-filter in ERPLAB (Lopez-Calderon and Luck, 2014) was used to remove electric-interference from the 50Hz-line; then the signal was band-pass filtered (1-70Hz for the 1st study and 1-40Hz for the 2nd study) using zero-phase shift-forward and reverse-IIR Butterworth-filter.
EEG data were manually inspected and non-brain related artifacts such as muscle contractions and movement-related artifacts were removed. This data cleaning procedure resulted in an average 91.7 (SD=18.9) and 87.9 (SD=20.77) seconds of data for controls and patients with schizophrenia, respectively. Additionally, an Independent Component Analysis (ICA)-based method was used with the ADJUST toolbox to eliminate the remaining muscle and eye-movement related artifacts
Analysis of gamma activity was performed on 60 seconds of artefact-free EEG data, based on its reliability in resting state EEG power spectra. For microstate segmentation, we used 40 seconds of artifact-free EEG data since earlier studies showed that this meets the quality requirements for the microstate analysis. In addition, we reduced the sampling rate to 128 Hz.

Based on the corrected EEG data, FFT was performed to extract absolute power applying the Welch's method, using 2 seconds, 75% overlap window and 0.5Hz frequency bin resolution. Gamma band power was calculated in 31-48Hz, by the summation of absolute power on frequency bins within the specified frequency range. Base-10 log transform was computed for the summed frequency band, which yielded a better approximation of normal distribution compared to the raw data.

For microstate segmentation, we adopted the standard procedure from an earlier work, where the modified K-mean clustering algorithm was used. The first step was a data reduction, where we selected those timepoints where the highest signal-to-noise ratio (SNR) can be achieved. Global Field Power (GFP) is good measure of SNR, derived as standard deviation of all channels at each timepoint. Since GFP shows oscillatory and scalp topographies that remain stable around GFP peaks, these timepoints were used further by the algorithm. Modified K-mean clustering represents an iterative algorithm, and for the first iteration, we randomly selected *n* time points for the initial template maps. In each iteration, we derived the spatial correlation of template maps with the spatial topography at each time point and marked the time point with the label of the most highly correlated template map. At the end of the

iteration, we derived new template maps based on the time points labeled with the same template maps. In the subsequent iteration, we used the new template maps. This iterative algorithm reaches a point where there is no change in the labels of the time points, thus no new maps can be derived.

The final maps can be quantified with global explained variance (GEV), which is a measure of how well the final derived spatial topographies can explain the variance of the raw topographical data. GEV values range from 0.58 to 0.84 in prior EEG microstate studies (Michel and Koenig, 2018). Since the initial template maps were selected randomly in the first iteration, we repeated this procedure for hundreds to thousands of times. The iteration where the highest GEV was achieved will serve for microstate classes for that individual subject. Based on previous literature, n = 4 microstate classes were used

In the third step, we derived the group average microstate maps from individual subjects using K-mean clustering. The four microstate maps of each individual in a group were concatenated, which resulted in a series of topography maps of individuals. To obtain the group-averaged microstate maps, we conducted K-mean clustering with the same iterative procedure (as in the second step) on this concatenated dataset. This resulted in the group-averaged microstate maps. These maps served for “back fitting”, where each time (sampling) point of

the pre-processed EEG was spatially correlated with each map and the given time point was labeled based on the most correlated map. The result is a sequence of microstate labels.

The sequence of microstates contains information on the occurrence of the series of microstate classes and serves as a basis to obtain various measures/features of microstate characteristics. The measures/features that are used in most of the EEG microstate studies include average duration, occurrence per second and total coverage of time for each microstate class. Since EEG microstates do not overlap with each other; the transition probability from one state to another can also be quantified and used as additional measures of EEG microstates. We used the Microstate toolbox, an extension to Matlab EEGlab toolbox, for the computations.

RESULTS

I. study

There were no significant between group differences in terms of age (F=2.66; p=0.1), gender (Chi2=2.27; p=0.13) and education level (Chi2=0.81; p=0.36). Nonetheless, we used these characteristics as covariates to adjust for their potential confounding.

Our findings indicated elevated gamma activity in patients with schizophrenia at rest. In terms of nominal significance, 118 out of 256 channels showed elevated gamma power in patients with schizophrenia as compared to healthy controls. A total of 78 channels of these remained significant after correction for multiple comparisons. The group differences emerged in two topographical areas, enclosing a fronto-central and posterior cluster. The fronto-central cluster comprised 29 channels (minimum nominal F-value=6.11; p=0.0147; maximum nominal F=15.89; p=0.0001). The posterior cluster comprised 49 channels (minimum F nominal value=7.15; p=0.0085; maximum nominal F=18.02; p<0.0001).

We performed additional analyses based on the clusters of channels in fronto-central and posterior regions. We found a significant interaction effect in three PANSS measures (total score, negative and hostility factor) in the posterior region

between the scalp location and symptom severity, which indicated that increase in gamma activity with symptom severity varied across the channels in the posterior scalp location ($F=2.38; p<0.0001$, $F=1.64$; $p<0.0037$ and $F=3.86$; $p<0.0001$, respectively). Based on these interactions, we further investigated which channels in this scalp area contributed significantly to the associations, and found that these channels were located over the left occipital cortex. Higher symptom severity, as measured by the PANSS total score, showed significant relationship with increased gamma power in 13 (out of 49) channels. We further analysed the five factors of PANSS, and found that higher negative and hostility factor scores showed significant relationship with increased gamma power in 11 and 15 (out of 49) channels, respectively.

We found no significant relationship between gamma power and CPZ equivalent dosages.

II. study

There were no significant between-group differences in terms of age ($F=2.66$; $p=0.06$), gender ($Chi^2=2.27$; $p=0.06$) and education level ($Chi^2=0.81$; $p=0.29$). Nonetheless, we used these characteristics as covariates to adjust for their potential confounding.

K-mean clustering algorithm provided four microstate classes for both groups where the average global explained variance was 77.8% (7.2% s.d.) and 75.6% (9.4% s.d.) for healthy controls and patients with schizophrenia, respectively, with no significant difference between study groups. In both groups, the four microstate topographies were similar to those that were previously identified in the literature. Each microstate classes characterized by the mean duration, occurrence per second, full coverage of time (yielding 12 features), as well as by the transition probabilities between classes (which included 12 more features), altogether a total of 24 features.

We used ANCOVA with covariates including age, gender, education level; moreover, the false discovery rate approach was used for statistical adjustment, due to the multiple comparisons. We found 14 features that show a significant difference between patients with schizophrenia and healthy controls. In particular, the occurrence and coverage of microstate class A ($F=2.9$, $p<0.05$; $F=2.37$, $p<0.05$) and D ($F=2.59$, $p<0.05$; $F=2.42$, $p<0.05$) were increased and the duration, occurrence, and coverage of the microstate class B ($F=4.87$, $p<0.05$; $F=5.96$, $p<0.05$; $F=5.47$, $p<0.05$) were decreased in patients with schizophrenia compared to healthy controls. We found no difference in the general properties of microstate class C.

The transition probability to class B ($F=5.39$, $p<0.05$; $F=5.85$, $p<0.05$; $F=9.63$, $p<0.05$) was significantly decreased in patients with schizophrenia, whereas from class C and D to class A ($F=3.1$, $p<0.05$; $F=3.23$, $p<0.05$) and from class A and C to class D ($F=2.97$, $p<0.05$; $F=2.75$, $p<0.05$) were significantly increased in patients with schizophrenia compared to healthy controls.

In the factor analysis, 3 factors achieved an eigenvalue that exceeded the a priori preset threshold of 1. The 3 factors contained 22 variables. Each factor was named based on the variables that the factor comprised ("A", "B" and "CD" factors). In the canonical analysis, the variables belonging to each factor were replaced by one variable, therefore we were able to characterize the subjects with 3 variables, which were used in SVM.

Our classification model was able to differentiate patients with schizophrenia from healthy controls with greater accuracy than random classification (mean AUC = 0.813, 95% CI = 0.812-0.814), as the AUC was larger than 0.5 (which would be in a model with randomly classification).

We used the three canonical variables for the classification. ROC curve analysis was used to delineate the potential diagnostic utility of the feature set. We achieved the best AUC with a value of 0.84 (accuracy: 82.7%, sensitivity/specificity:

82.67%/81.43%). The mean accuracy and the standard deviation for the 100 repetitions of CV was 81.82% (SD=0.95), while the mean sensitivity and specificity were 81.34% (SD=0.67%) and 79.02% (SD=1.63%), respectively.

To assess the impact of feature selection, we conducted a further analysis by including all features from all factors (i.e., 22 features) in SVM for classification purposes. The highest AUC value that was achieved was 0.81 (accuracy: 78.7%, sensitivity/specificity: 76%/81.4%). The mean accuracy and the standard deviation for the 100 repetitions of CV was 75.06 % (SD=1.36), while the mean sensitivity and specificity were 70.81% (SD=2.07%) and 79.61% (SD=1.46%), respectively. Thus, with our feature selection algorithm, the SVM approach yielded slightly higher AUC and accuracy than the analysis which was based on all features from factors. Sensitivity increased markedly (from 70.81% to 81.34), while specificity showed less increase (from 79.61% to 79.02%) as compared to the results that were based on the full feature set (all features from factors).

CONCLUSIONS

The two studies presented in the dissertation are related to the neurobiological abnormalities in schizophrenia summarized in the literature review and the disease models based on them. Overall, our results are consistent with literature data and confirm the existence of cerebral network dysfunction in schizophrenia. There is a close relationship between resting networks and task-specific networks, hence impairment of resting networks may be associated with altered perceptual, cognitive, and memory functions.

The change in gamma activity identified in the first study indicates differences in gamma oscillations in the cortex. The consequence of the alteration may lead to a loss of synchronization of the networks over time, which may result in information distortion and thereby impairment to the affected function. In frontal regions, aberrant resting gamma performance may be the basis for neurocognitive abnormalities in schizophrenia. Parietal regions, together with frontal areas, perform hetero-modal association tasks that are impaired in schizophrenic patients. In the occipital areas, increased gamma can be interpreted as cortical noise, which affects visual perception processes, and it is known that disturbed gamma

oscillations have been observed in patients with schizophrenia in the visual Gestalt paradigm.

In the second study, we used the characteristics of EEG microstates in a machine learning model to classify schizophrenic patients and healthy controls. Differences identified on the basis of the microstate characteristics proved to be sufficient for good accuracy in classification. Several correlations between microstates and resting networks have been identified in the literature. Differences in micro-states may indicate impairments in the dynamics and temporal alignment of brain networks. Network damage, in turn, can lead to impairment in terms of functionality.

The results of the two studies may indicate disturbances in the functioning of brain networks. While gamma activity is a measure of the synchronous activity of networks, the characteristics of microstates provide information about the structural and dynamic aspects of several brain subnets. Furthermore, they may help expand the machine learning model used in the second study, i.e. to develop a more effective classification model using the differences measured in gamma activity.

Differences in neurotransmitter systems detailed in the literature review lead to damage to synaptic network connections, which can lead to differences in macroscopic

brain-networks. Our studies offer additional data for the dysconnectivity hypothesis of schizophrenia and for theories based on the disruption of the reduced signal-to-noise ratio and the stimulus-inhibitor balance.

PUBLICATIONS RELATED TO THE DISSERTATION

Baradits, M., B. Kakuszi, S. Balint, M. Fullajtar, L. Mod, I. Bitter and P. Czobor (2019). "Alterations in resting-state gamma activity in patients with schizophrenia: a high-density EEG study." Eur Arch Psychiatry Clin Neurosci 269(4): 429-437.

Baradits, M., Bitter, I., Czobor, P., 2020. Multivariate patterns of EEG microstate parameters and their role in the discrimination of patients with schizophrenia from healthy controls. Psychiatry Res 288, 112938.

OTHER PUBLICATION

Czobor P, Kakuszi B., **Baradits M.**, Bálint S., Bitter I. (2017). "Új elektrofiziológiai vizsgálatok a pszichiátriában" Orvostovábbképző Szemle XXIV. évf. 1. Szám