

VALPROATE TREATMENT NORMALIZES EEG FUNCTIONAL CONNECTIVITY IN
SUCCESSFULLY TREATED IDIOPATHIC GENERALIZED EPILEPSY PATIENTS

¹Clemens B., MD, PhD; ²Puskás S., MD, PhD; ³Besenyei M., MD; ¹Kovács N.Zs.; ⁴Spisák T.;
⁴Kis S.A., PhD; ⁴Emri M., PhD; ⁵Hollódy K., MD, PhD; ⁶Fogarasi A., MD, PhD; ⁷Kondákor I,
MD, PhD; ²Fekete I., MD, PhD.

¹ Kenézy Gyula County Hospital, Department of Neurology, Debrecen, Hungary

² University of Debrecen, Medical Center, Department of Neurology, Debrecen, Hungary

³ University of Debrecen, Medical Center, Department of Pediatrics, Debrecen, Hungary

⁴ University of Debrecen, Department of Nuclear Medicine, Debrecen, Hungary

⁵ University of Pécs, Department of Pediatrics, Pécs, Hungary

⁶ Epilepsy Center, Bethesda Children's Hospital, Budapest, Hungary

⁷ Balassa János Hospital, Department of Neurology, Szekszárd, Hungary

Corresponding author: Szilvia Puskás, MD, PhD.

University of Debrecen, Medical Center, Department of Neurology,
Debrecen, Hungary

Móricz Zsigmond krt. 22.

4032 Debrecen

HUNGARY

TEL: ++36 52 255255

Fax: ++36 52 255590

E-mail: szilvia.puskas@yahoo.com

Running title: Valproate normalizes EEG functional connectivity

Abstract

Aim. To investigate the effect of chronic VPA treatment of EEG functional connectivity in successfully treated idiopathic generalized epilepsy (IGE) patients.

Patients and Methods. 19-channel waking, resting-state EEG records of 26 IGE patients were analyzed before treatment (IGE) and after the 90th day of treatment (VPA), in seizure-free condition. Three minutes of artifact-free EEG background activity (without epileptiform potentials) was analyzed for each patient in both conditions. A group of 26 age-matched healthy normative control persons (NC) was analyzed in the same way. All the EEG samples were processed to LORETA (Low Resolution Electromagnetic Tomography) to localize multiple distributed sources of EEG activity. Current source density time series were generated for 33 regions of interest (ROI) in each hemisphere for four frequency bands. Pearson correlation coefficients (R) were computed between all ROIs in each hemisphere, for four bands across the investigated samples. R values corresponded to intrahemispheric, cortico-cortical functional EEG connectivity (EEGfC). Group and condition differences were analyzed by statistical parametric network method.

Main results. ($p < 0.05$, corrected for multiple comparisons). 1. The untreated IGE group showed increased EEGfC in the delta and theta bands, and decreased EEGfC in the alpha band (as compared to the NC group). 2. VPA treatment normalized EEGfC in the delta, theta and alpha bands. 3. Degree of normalization depended on frequency band and cortical region.

Conclusions. VPA treatment normalizes EEGfC in IGE patients.

Keywords

Idiopathic generalized epilepsy, valproate, EEG functional connectivity

Introduction

Valproate (VPA) is an effective antiepileptic drug for idiopathic generalized epilepsy (IGE) syndromes. Its anticonvulsive mechanisms were thoroughly investigated at the molecular and cellular levels. Classic pharmacological research demonstrated that VPA binds to several neuronal and glial targets and modifies electric activity of these cells (Cotariu et al., 1990; Löscher, 2002; Capek and Esplin, 1990). However, the results do not explain the full therapeutic effect of VPA. The best argument for this is the so-called delayed or carryover effect of VPA (Löscher, 2002) that contributes to its superior therapeutic efficacy in IGE (Nicolson et al., 2004). The delayed effect suggests that VPA causes re-arrangement of cerebral structure and/or function, which protects against seizures and is independent of the momentary serum level of the drug (Burr et al., 1984; Stefan et al., 1984). Recently, a few other studies supported the enduring effect of VPA on cerebral connectivity, which may give rise to beneficial or adverse effects. VPA inhibits effective connectivity among motor areas in healthy volunteers (Li et al., 2011). VPA alters expression of multiple genes in the CNS in rats (Fukuchi et al., 2009) and epilepsy patients as well (Tang et al., 2004). VPA modifies cortical excitability by modifying neuron-glia relationship (Wang et al., 2012), influences myelin production, repairs and alters neuronal connectivity (Rosenzweig et al., 2012). IGEs are increasingly realized as network disorders, so it is reasonable to suppose that VPA modifies abnormal cerebral connectivity.

The aim of this study was to investigate the effect of chronic VPA administration on functional EEG connectivity (EEGfC) in IGE patients. The key-lock principle of pharmacology-EEG (Saletu et al., 2002) suggests that if epilepsy results from abnormal cortical function, normalization of that function results in clinical improvement. We have tested the hypothesis that VPA reverses abnormal resting-state intrahemispheric, cortico-cortical EEGfC in successfully treated IGE patients. Targeting this part of cerebral connectivity is justified because abnormal intrahemispheric connectivity is the neurophysiological basis of seizure-prone state and ictogenesis in experimental models (Timofeev and Steriade, 2004) and IGE syndromes as well (Holmes et al., 2004, 2010; Clemens et al., 2013).

2. Patients and methods

2.1. Patients and control persons

The study design was approved by the Local Research Ethics Committee of Kenézy Gyula County Hospital, Debrecen, Hungary. All unmedicated IGE patients, who visited one of the collaborating epilepsy outpatient services, were potential candidates for the study. Patients with recent-onset IGE were diagnosed according to generally accepted criteria (Panayiotopoulos, 2005). No diagnostic procedure was indicated, missed, or postponed for study purposes only. Therapeutic decision has done after correct diagnosis was stated. The patients were informed about risks and benefits of the drug treatment, with special reference

to VPA-related risk for females of childbearing age. VPA was not the drug of first choice for those who desired to be pregnant within a few years. Also patients who had a well-documented, longer history of IGE but did not take medication for any reason were potentially eligible for the study. Risk-benefit estimation was exposed to them as well and individual experience with prior drug treatment was taken into consideration.

Inclusion criteria were: first seizure after the fifth year of life; clinically and electrographically unequivocal findings indicating one of the common IGE syndromes: idiopathic childhood absence or juvenile absence epilepsy (ABS), juvenile myoclonic epilepsy (JME), epilepsy with generalized tonic-clonic seizures exclusively (GTC); the decision to treat the patient with VPA monotherapy. Exclusion criteria were: significant neurological or psychiatric comorbidity, metabolic disorders, alcohol or substance abuse and any other medical condition that is known to significantly modify EEG activity. Patients having a generalized tonic-clonic seizure in the five days prior EEG investigation were excluded. 26 patients entered the study (ABS = 14, JME = 7, GTC = 5). Age-and sex distribution were: 11 males, 15 females, 7-54 years of age, mean age: 17.2 years). 17 patients had recent-onset IGE, 4 patients had long-lasting IGE, disease onset was uncertain in 5 patients. Baseline clinical and EEG evaluations were carried out at entry visit. Initial daily dose of VPA was 300 to 500 mg. The dose was increased until seizure freedom was reached. The final daily dose of VPA was 300 to 1500 mg. The second EEG was recorded 3 month after the first one because the initial, transient EEG effects of the drug disappear by this time (Sannita et al, 1989). The patients' general and neurological condition did not change from initial to control evaluation. No patient reported complaints indicating neurotoxicity.

To evaluate the baseline EEGfC abnormality in the patient group, a group of healthy, normal control persons (NC) was created. Each patient was matched to a NC person of same age and sex. Mean age for the NC group was 16.9 years.

2.2. EEG recording and sample selection

EEG recordings were carried out in the morning, after a night of sufficient sleep, in a semi-isolated room, with the same type of digital equipment, by trained personnel. Silver-silver chloride electrodes were placed according to the 19 sites of the 10-20 system and the earlobes, fixed by appropriate adhesive and conductive gel. Impedances did not exceed 10 kOhm. EEG was recorded against Fpz sampling reference and recomputed against a mathematical linked ears reference. Additional bipolar derivations were used to differentiate between EEG and eye movement potentials and to detect myogenic activity. EEG filters were set at 0.1 and 33.6 Hz. Sampling rate was 256 per second, on-line digitization was 12 bit. 30 minutes EEG was recorded in the waking-relaxed, eyes-closed condition. The state of vigilance was controlled by the EEG technician who gently aroused the patient when the posterior alpha rhythm disappeared. Thirty 2-second epochs were selected for analysis, according to our standard epoch selection protocol: 1. the presence of continuous alpha activity with voltage maximum in posterior regions, 2. the absence of artifacts, epileptiform potentials and other nonstationary elements, 3. the absence of patterns indicating

drowsiness or arousal. As to prevent unwanted selection bias, epoch selection was carried out by one of us who was blinded to the condition of the patient (untreated vs. treated) in which EEG was recorded. Final visual control of the selected epochs was carried out by the senior author as to ensure the quality of the sample. This electrographic definition of the relaxed-waking state refers to a narrow range of vigilance (Bente, 1979). Two reproducibility measures were used to minimize the effect of short- and long-term variability within the samples. Only samples with at least 95 percent of average split-half reliability and test-retest reliability (across the 19 channels) entered further analysis. This level of reliability was reached in all samples, so no records were excluded from analysis.

2.3. Quantitative EEG analyses

Quantitative EEG analyses were carried out by means of the NeuroGuide Deluxe (Version 2.7.4) software (Thatcher, www.appliedneuroscience.com) and joined software as specified below.

2.3.1. Localization of the sources of EEG activity

Low Resolution Electromagnetic Tomography (LORETA) is a method for localizing multiple distributed sources of EEG activity in the three-dimensional space (Pascual-Marqui et al., 1994). LORETA demonstrates the synchronously activated neuronal populations underlying EEG activity by computing their cortical localization from the scalp distribution of the electric field. The LORETA inverse solution is based on the "smoothness" assumption, which means that neighbouring EEG generators produce maximally correlated activity in terms of orientation and strength. The smoothness assumption is based on neuroanatomical and electrophysiological constraints. The brain compartment of this model is restricted to the cortical grey matter and hippocampus. The grey matter compartment is divided into 2394 voxels, which allows a spatial resolution of 7 millimeters. Localization of voxels is based on coordinates of the Talairach Brain Atlas (Talairach and Tournoux, 1988). LORETA computes current source density (CSD; expressed as Amperes / meters squared) for each voxel, briefly called "LORETA activity" in the literature. The consistency of LORETA with physiology and localization has been validated by several authors (Pascual-Marqui, 2002). Importantly, LORETA based on 16 to 28 channel EEG recordings localizes the sources of maximum EEG activity concordantly to the reference localization methods (positron emission tomography, functional MRI, MRI diffusion spectral imaging, intracranial EEG recordings) when the electrical source distribution is "neurophysiologically smooth" (Pascual-Marqui et al., 2002; Oakes et al., 2004). This is the case in the epileptic cortex where abnormally synchronized activity is distributed across a lot of cortical columns and even greater cortical areas via the dense network of intracortical connections (Chagnac-Amitai and Connors, 1989). Furthermore, LORETA localization with this number of electrodes is reliable when delta, theta, alpha and lower beta frequencies that subserve global integration of higher cerebral functions and penetrate the entire cerebral volume are investigated (Babiloni et al., 2006).

2.3.2. Analysis of resting-state EEG functional connectivity

Correlating the activity of LORETA-localized sources (CSD = current source density) is a useful alternative to correlate quantitative EEG variables measured at scalp electrodes and offers a deeper understanding of intrahemispheric cortico-cortical connectivity (Thatcher et al., 2007; Schoffelen and Gross 2009). LORETA Source Correlation (LSC) analysis means computing the temporal covariance or correlation of LORETA-defined CSD between two cortical areas (region of interest, ROI), across successive 2-second epochs over the investigated sample. The Pearson product correlation coefficient (r) is a valid measure of oscillator coupling, especially when a relatively long interval of time is analyzed, as in this study. Authors who compared the sensitivity and reliability of several methods concluded that Pearson correlation is a robust method being sensitive to all the investigated coupling parameters and does not require specific assumptions about the model (Wendling et al., 2009). Given the 19 scalp electrodes, the effect of the point spread on CSD estimates was minimized by clustering hundreds of nearby voxels into 33 ROIs in each hemisphere. Fig. 1. shows the flowchart of computing asymmetric EEGfC matrices (Thatcher et al., 2007, Clemens et al., 2013). This figure indicates that two correlation coefficients characterize the EEGfC between two ROIs, producing asymmetric connectivity matrices.

In order to avoid the asymmetry we have generated a set of symmetric source correlation matrices from the average of the two correlation coefficients between ROIs: R_{gthbs} , stand for group ($g \in \{vpa, nc\}$), treatment ($t \in \{before, after\}$), hemisphere ($h \in \{LH, RH\}$), band ($b \in \{delta, theta, alpha, beta\}$) and subject indices ($s = 1 \dots N_g$) respectively. A single element of an R_{gthbs} matrix was denoted by r_{gthbs}^e (where e represents a connection between two regions). The number of rows and columns are equal with the number of ROIs ($N=33$) and with the number of correlation coefficients $M=N(N-1)/2$ ($M=528$). All analyses were based on broad-band results of four frequency bands (delta: 0.5-3.0 Hz, theta: 3.5-7.0 Hz; alpha: 7.5-12.0 Hz; beta: 12.5-25.0 Hz).

2.3.3. Statistical inference of connections

Statistical parametric network (SPN) terminology has been introduced (Ginestet et al., 2011). In our study we generated population and treatment-related differential SPNs which provide a statistical method to investigate differences of connections. SPNs were calculated from R_{gthbs} matrices, using M mixed-effect models:

$$r_{gthbs}^e = X_{gthbb}^e \beta^e + Z_s^e b_s^e + \varepsilon_{gthbs}^e$$

where r are the correlation coefficients of interest, β is a vector of fixed effect (group, treatment, hemisphere and band) which does not vary over subjects, b is the subject-specific random effects (subject, age-group) and ε are the residuals.

In this study four age-groups were defined: child (less than 10 years), teenager (between 10 and 20 years), young adult (between 20 and 40 years) and middle aged (more than 40 years). The matrices X and Z contain the fixed-effect and the random-effect components of the linear model. The group and the treatment effects for all bands were evaluated by a post-hoc Tukey test which produced t_{ij} Student t-values for all edges, hemispheres and bands. These t-values were stored in $N \times N$ SPN matrices for visualization. Statistically significant differences of EEGfC at corrected $p < 0.05$ level were thresholded by local false discovery rate (FDR) for multiple comparison (Efron 2004, 2007).

2.3.5. Software

For EEG sample selection and quantitative EEG analyses, we used NeuroGuide Deluxe (Version 2.7.7.; www.appliedneuroscience.com), LORETA (Pascual-Marqui et al., 1994) and LORETA Source Correlation (Thatcher et al., 2007). SPN were evaluated by home-developed BrainNetTools software. For the visualization of differences between significant connections BrainCON (www.minipetct.com/braincon) was used (Spisák et al., 2013).

2.3.6. Interpretation of the results in the topographic domain

LORETA localization is the key feature of all the subsequent analyses. Because its localization accuracy is limited, activity of small ROIs may be falsely localized. However, a prior study demonstrated that VPA alters LORETA activity in widespread cortical regions (Clemens et al., 2007), so very precise localization was presumably not essential in this study. We discuss EEGfC results at sub-lobar, lobar and network levels, which is a usual approach in the neuroimaging literature (Anderson and Hamandi, 2011).

3. Results

3.1. Untreated IGE group vs. NC group

Statistically significant differences between the untreated IGE and the NC groups demonstrate baseline EEGfC abnormality in IGE (Fig. 2., top row). The untreated IGE group showed overall greater *delta* and *theta* EEGfC than the NC group. No striking asymmetry and no opposite sense differences emerged in these bands. The untreated IGE group showed greater *alpha* EEGfC among frontal ROIs including the uncus (a temporal ROI) and less *alpha* EEGfC across the rest of the cortex as compared to the NC group. Greater *beta* EEGfC emerged among right frontal ROIs in the untreated patients than in the NC group.

3.2. Treated vs. untreated conditions of the IGE group

Comparison of the treated and untreated IGE conditions shows drug effects (Fig. 2., middle row). VPA decreased *delta* EEGfC between most ROIs except the left and right paracentral lobules, cingulate gyri, and the right parietal lobe. VPA decreased *theta* EEGfC in the anterior parts of the hemispheres and increased *theta* EEGfC between the paracentral lobules and the parietal ROIs bilaterally. VPA increased *alpha* EEGfC between most ROIs but did not affect EEGfC between frontal ROIs (where increased EEGfC was found in the untreated patients) and even decreased EEGfC in the left lateral frontal area. VPA increased *beta* EEGfC between right frontal and temporal ROIs, and three increased EEGfC values were found in the left hemisphere.

3.3. Treated IGE patients vs. NC group

Comparison of the treated IGE group and the NC groups highlights the degree of the normalization in the four bands (Fig. 2., bottom row). Greater overall *delta* and *theta* EEGfC were found in the treated condition of the IGE group as compared to the NC group. Greater *alpha* and *beta* EEGfC were found between right frontal ROIs in the treated IGE group as compared to the NC group. Less *alpha* EEGfC was found in a few, fronto-parietal and fronto-occipital connections in the treated IGE group as compared to the NC group. Greater *alpha* and *beta* EEGfC in the right frontal lobe (and in a few other connections) were found in the treated IGE group than in the NC group.

3.4. VPA-related EEGfC normalization

VPA treatment decreased abnormally high EEGfC and increased abnormally low EEGfC between most, but not all, pairs of ROIs in the delta, theta and alpha bands. VPA-related shift of a single abnormal EEGfC value towards the normative value means EEGfC normalization and is defined as follows. "Full normalization" of an abnormal connection means: abnormal baseline value + statistically significant difference between the untreated and treated conditions + no statistically significant difference between the treated condition and the NC group. "Partial normalization" means: abnormal baseline value + statistically significant difference between the untreated and treated conditions + statistically significant difference between the treated condition and the NC group. "No normalization" is defined as: abnormal baseline value + no statistically significant difference between the untreated and treated conditions + statistically significant difference between the treated condition and the NC group.

With this logic in mind, VPA treatment fully normalized *delta* EEGfC between several frontal ROIs bilaterally (medial frontal gyrus, orbital gyrus, straight gyrus, subcallosal and extra-nuclear areas) and the uncus. Lack of abnormal EEGfC in this area is better demonstrable in sagittal view (Fig. 3). VPA partially normalized *delta* EEGfC between ROIs in the rest of the cortex except the right parietal area. VPA partially normalized EEGfC between anterior ROIs in the *theta* band. Full EEGfC normalization occurred in most long-range connections in the *alpha* band. On the other hand, there was no normalization of

abnormally increased baseline *alpha* EEGfC between frontal ROIs in the right hemisphere, while full or partial normalization occurred in the connections of the left frontal area. No EEGfC normalization occurred in the *beta* band. More increased EEGfC values were found in the treated than in the baseline condition.

4. Discussion

As far as is known, this is the first study to investigate the effect of therapeutic VPA administration on EEGfC in IGE patients. The findings confirmed the hypothesis that VPA reverses abnormal resting-state intrahemispheric, cortico-cortical EEGfC in successfully treated IGE patients. This means that VPA shifted most baseline EEGfC abnormalities towards normative values. However, the degree of normalization depended on frequency bands, cortical regions and the magnitude of baseline EEGfC abnormality.

4.1. Changes in the delta and theta bands

Delta, theta, alpha and beta bands reflect natural classes of brain oscillations (Buzsáki and Draghoun, 2004) generated by "selectively distributed neuronal systems" (Basar and Schürmann, 1999). Thus, it is not surprising that VPA causes band-specific changes in electrical activity of the cortex. In this study we demonstrated decrease and normalization of EEGfC in the delta and theta bands. This finding is reminiscent of activity-dependent, normalizing effect of VPA on delta and theta spectral power in IGE patients. The effect was independent of the daily dose and serum level of VPA, so we concluded that it reflects enduring changes within the CNS (Clemens, 2008). Local EEG synchronization (expressed as voltage of the signal or CSD) depends on the dense intracortical circuitry modified by subcortical inputs (Nunez, 1995). Remote EEG synchronization (EEGfC, defined in Section 2.3.2) means synchronized fluctuations of CSD in two great neuronal ensembles, in other words, two cortical areas. This effect is mediated by short- and long distance association fibers (Thatcher et al., 2007) and other, yet not fully known mechanisms (Magineanu, 2010). Estimates of local and remote EEG synchronization may change independently. However, VPA decreases and normalizes delta and theta activity at both spatial scales, which is in accord with the complexity of drug effects on the CNS (Magineanu, 2012).

Little is known about the relationship of band-related EEGfC and epilepsy. Epilepsy patients show increased EEGfC in the delta band in the interictal state (Horstmann et al., 2010). Recently, increased theta band synchronization was reported in epilepsy (Douw et al., 2010). Increased delta and theta EEGfC were found in untreated JME patients in the interictal state (Clemens et al., 2013). These findings suggest that increased remote EEG synchronization in the slow frequency bands (delta and/or theta) is somehow related to epilepsy and is independent of the syndrome. An experimental study demonstrated that increased phase stability (another measure of remote EEG synchronization) in the theta band is a neurophysiological corollary of epileptogenesis and persists in the epileptic state (Ge et al., 2013). With this evidence in mind we suggest that VPA-related decrease of delta

and/ or theta EEGfC may be related to successful treatment. This argumentation is in accord with the key-lock principle of pharmaco-EEG (Saletu et al., 2002). However, alternative solutions cannot be excluded. VPA may cause connectivity changes in the CNS that do not modify seizure control (Rosenzweig et al., 2012). Demonstrating the lack of significant EEGfC drug effects in VPA-treated nonresponder patients might support the relationship between delta-theta EEGfC normalization and therapeutic efficacy.

4.2. Changes in the alpha and beta bands

VPA increased and normalized alpha band EEGfC between most ROIs that belong to the "classic" alpha system (Basar et al., 1989). Cortical generators of this system are mostly localized in posterior areas but some alpha activity may be recorded over the rest of the cortex. As far as is known, epileptogenesis and the epileptic condition do not significantly affect the alpha system, so the significance of alpha EEGfC normalization remains hidden.

VPA did not normalize baseline alpha EEGfC among right frontal ROIs and partially normalized alpha EEGfC in the left frontal area. This unexpected finding demonstrated that drug effect may differentiate between neuronal systems that operate at the same frequency band at the same time. Our results are consistent with the opinion that the medial frontal alpha rhythm is not generated by the "classic" alpha system (Röschke et al., 1997; Feshchenko et al., 2001).

VPA did not affect beta band EEGfC in the majority of connections. Treated condition of the IGE group showed somewhat more abnormal beta connectivity values than the untreated condition. This drug effect has no explanation at present.

4.3. Limitations of the broad-band approach

Besides the band-specific trends of EEGfC changes a few, topographically sporadic VPA effects occurred in this study (Fig. 2, middle row). These findings may be explained by inter-individual variability of electrophysiological results. Individual variability may cause blurring of the broad band findings in group studies. Decreased left fronto-lateral alpha EEGfC actually might belong to strongly decreased theta EEGfC in the same region. Increased cingular-parietal theta EEGfC is topographically similar to increased EEGfC in the neighbouring alpha band. Our unpublished personal experience with parallel broad band and very narrow band (VNB, 1 Hz bandwidth) analyses shows that strong topographical broad band patterns may intrude into neighbouring VNB of the next broad band. So, there is no obligatory need to search after neurophysiological basis and clinical importance of findings that seem to intrude from one broad band to the neighbouring one. If the finding seems to be important, narrow band analysis can highlight its exact spectral distribution (Szava et al., 1994).

4.4. Regional differences of the VPA effect

Partial normalization of delta EEGfC was found between most ROIs but full normalization occurred in the medial-basal frontal area and the uncus in the temporal lobe. Potential importance of this finding is that the medial-basal frontal cortex is the seizure onset zone in ABS (Holmes et al., 2004) and JME (Holmes et al., 2010).

Lack of VPA effect in the delta band and right cingular-parietal area was a peculiar finding. Major clinical and EEG manifestations are usually symmetrical in IGE syndromes. Whether the reported asymmetrical drug effect is specific to epilepsy or IGE or reflect general response of the human brain to repeated VPA administration remains hidden. We are not aware of any topographical EEG analysis of VPA effects in healthy persons and patient groups.

VPA significantly decreased theta EEGfC between anterior but not posterior ROIs. This finding harmonizes with VPA-related decrease of local CSD in frontal but not posterior areas in IGE (Clemens et al., 2007). Baseline theta EEGfC abnormality was greater between anterior than between posterior ROIs. Furthermore, also neuroimaging studies found the main structural and functional abnormalities in the anterior parts of the brain in IGE syndromes (Anderson and Hamandi, 2011).

References

Anderson, J., Hamandi, K., 2011. Understanding juvenile myoclonic epilepsy: Contributions from neuroimaging. *Epilepsy Res.* 94,3, 127-137.

Babiloni, B., Binetti, G., Cassarino, A., Dal Forno, G., Del Percio, C., Ferreri, F., Frisoni, G., Galderisi, S., Hirata, K., Lanuzza, B., Miniussi, C., Mucci, A., Nobili, F., Rodriguez, G., Romai, G.L., Rossini, P.M., 2006. Sources of cortical rhythms in adults during physiological aging: a multicentric EEG study. *Hum. Brain Mapp.* 27, 162-172.

Bente, D., 1979. Vigilance and evaluation of psychotropic drug effect on EEG. *Pharmacopsychiatry* 12, 137-147.

Burr, W., Fröscher, W., Hoffmann, F., Stefan, H., 1984. Lack of significant correlation between circadian profiles of valproic acid serum levels and epileptiform electroencephalographic activity. *Ther. Drug Monitoring* 6, 179-181.

Buzsáki, G., Draguhn, A., 2004. Neuronal oscillations in cortical networks. *Science* 304, 1926-1929.

Basar, E., Schürmann, M., 1999. Brain functioning: Integrative Models, in: Basar, E., (Eds.), *Brain function and oscillations. Vol. II: Integrative brain function. Neurophysiology and cognitive processes.* Springer, pp. 393-406.

- Capek, R., Esplin, B., 1990. Mechanisms of anticonvulsant action of valproate. An overview and perspective, in: Avoli, É.M., Gloor, P., Kostopoulos, G., Naquet, R. (Eds.), *Generalised epilepsy. Neurobiological Approaches*. Birkhauser, Boston, pp. 436-459.
- Chagnac-Amitai, Y., Connors, B.W., 1989. Horizontal spread of synchronized activity in neocortex and its control by GABA-mediated inhibition, *J. Neurophysiol.* 61, 747-758.
- Clemens, B., 2008. Valproate decreases EEG synchronization in a use-dependent manner in idiopathic generalized epilepsy. *Seizure* 17, 224-233.
- Clemens, B., Bessenyey, M., Tóth, M., Kondákor, I., 2007. Valproate selectively reduces EEG activity in anterior parts of the cortex in patients with idiopathic generalized epilepsy. A low resolution electromagnetic tomography (LORETA) study. *Epilepsy Res.* 75, 186-191.
- Clemens, B., Puskás, S., Besenyey, M., Spisák, T., Kis, S.A., Hollódy, K., Fogarasi, A., Fekete, I., Emri, M., 2013. Neurophysiology of juvenile myoclonic epilepsy. EEG-based network and graph analysis of the interictal and immediate preictal states. *Epilepsy Res.* 106, 3, 357-369.
- Cotariu, D., Zaidman, J.L., Evans, S., 1990. Neurophysiological and biochemical changes evoked by valproic acid in the central nervous system. *Prog. Neurobiol.* 34, 343-354.
- Douw, L., de Groot, M., van Dellen, E., Heimans, J.J., Ronner, H.E., Stam, C.J., Reijneveld, J.C., 2010. 'Functional connectivity' is a sensitive predictor of epilepsy diagnosis after the first seizure. *PLoS One* 5, e10839.
- Efron, B., 2004. Large-scale simultaneous hypothesis testing: the choice of a null hypothesis. *J. Amer. Stat. Assoc.* 99, 96-104.
- Efron, B., 2007. Correlation and Large-Scale Simultaneous Significance Testing. *Jour. Amer. Stat. Assoc.* 102, 93-103.
- Feshchenko, V.A., Reinsel, R.A., Veselis, R.A., 2001. Multiplicity of the alpha rhythm in normal humans. *J. Clin. Neurophysiol.* 18,4, 331-44.
- Fukuchi, M., Nii, T., Ishimaru, N., Minamino, A., Hara, D., Takasaki, I., Tabuchi, A., Tsuda, M., 2009. Valproic acid induces up- or down-regulation of gene expression responsible for the neuronal excitation and inhibition in rat cortical neurons through its epigenetic action. *Neurosci. Res.* 65, 35-43.
- Ginestet, C.E., Nichols, T.E., Bullmore, E.T., Simmons, A., 2011. Brain network analysis: separating cost from topology using cost-integration. *PLoS One* 6,7.

Ge, M., Wang, D., Dong, G., Guo, B., Cao, R., Sun, W., Zhang, J., Liu, H., 2013. Transient impact of spike on theta rhythm in temporal lobe epilepsy. *Experimental Neurology*. 250, 136-142.

Holmes, M.D., Brown, M., Tucker, D.M., 2004. Are "generalized" seizures truly generalized? Evidence of localized mesial frontal and frontopolar discharges in absence. *Epilepsia* 45, 1568-1579.

Holmes, M.D., Quiring, J., Tucker, D.M., 2010. Evidence that juvenile myoclonic epilepsy is a disorder of frontotemporal corticothalamic networks. *Neuroimage* 49, 80-93.

Horstmann, M.T., Bialonski, S., Noennig, N., Mai, H., Prusseit, J., Wellmer, J., Hinrichs, H., Lehnertz, K., 2010. State dependent properties of epileptic brain networks: comparative graph-theoretical analyses of simultaneously recorded EEG and MEG. *Clin. Neurophysiol.* 121, 172-185.

Li, X., Large, C.H., Ricc, R., Taylor, J.J., Nahas, Z., Bohnin, D.E., Motgan, P., George, M.S., 2011. Using interleaved magnetic stimulation/functional magnetic resonance imaging (fMRI) and dynamic causal modeling to understand the discrete circuit specific changes of medication: lamotrigine and valproic acid changes in motor and prefrontal effective connectivity. *Psychiatry Res.: Neuroim.* 194, 141-148.

Löscher, W., 2002. Basic pharmacology of valproate. A review after 35 years of clinical use for the treatment of epilepsy. *CNS Drugs* 16, 669-694.

Margineanu, D.G., 2010. Epileptic hypersynchrony revisited. *NeuroReport* 21, 963-967.

Margineanu, D.G. 2012. Systems biology impact on drug discovery. *Epilepsy Res.* 98,2-3, 104-15.

Nicolson, A., Appleton, R.E., Chadwick, D.W., Smith, D.F., 2004. The relationship between treatment with valproate, lamotrigine, topiramate and the prognosis of the idiopathic generalized epilepsies. *J. Neurol. Neurosurg. Psychiat.* 75, 75-79.

Nunez, P.L., 1995. Quantitative states of neocortex, in: Nunez, P.L., (Eds.), *Neocortical dynamics and human EEG rhythms*. Oxford University Press, pp.1-18.

Oakes, T.R., Pizzagalli, D.A., Hendrick, A.M., Horras, K.A., Larson, C.L., Abercrombie, H.C., Schaefer, S.M., Koger, J.V., Davidson, R.J., 2004. Functional coupling of simultaneous electrical and metabolic activity in the human brain. *Hum. Brain Mapp.* 21, 257-270.

Panayiotopoulos, C., 2005. Idiopathic generalized epilepsies, in: Panayiotopoulos C (Eds.), *The epilepsies. Seizures, syndromes, and management*. Springer, pp. 271-348.

Pascual-Marqui, R.D., Michel, C.M., Lehmann, D., 1994. Low resolution electromagnetic tomography. a new method for localizing electrical activity in the brain. *Int. J. Psychophysiol.* 18, 49-65.

Pascual-Marqui, R.D., 2002. Functional imaging with low-resolution brain electromagnetic tomography (LORETA): a review. *Meth. Find. Exp. Clin. Pharmacol.*, 24, 91-96.

Pascual-Marqui, R.D., Esslen, M., Kochi, K., Lehmann, D., 2002. Functional imaging with low-resolution brain electromagnetic tomography (LORETA): review, new comparisons, and new validation. *Jap. J. Clin. Neurophysiol.* 30, 81-94.

Rosenzweig, I., Vukadinovic, Z., Turner, A.J., Catani, M., 2012. Neuroconnectivity and valproic acid. The myelin hypothesis. *Neurosci. and Behavioral Reviews* 36, 1848-1856.

Röschke, J., Fell, J., Mann, K. 1997. Non-linear dynamics of alpha and theta rhythm: correlation dimension and Lyapunov exponents from healthy subject's spontaneous EEG. *Int. J. Psychophysiol.* 26, 251-261.

Saletu, B., Anderer, P., Saletu-Zyhlarz, G.M., Pascual-Marqui, R.D., 2002. EEG topography and tomography in diagnosis and treatment of mental disorders: evidence for a key-lock principle. *Methods Find. Exp. Clin. Pharmacol.* 24.

Sannita, W.G., Gervasio, L., Zagoni, P., 1989. Quantitative EEG effects and plasma concentration of sodium valproate: acute and long-term administration to epileptic patients. *Pharmacoelectroencephalography*; 22:231-235.

Schoffelen, J.M., Gross, J., 2009. Source connectivity analysis with MEG and EEG. *Hum. Brain Mapp.* 30, 1857-1865.

Spisák, T., Opposits, G., Kis, S.A., Pohubi, L., Jakab, A., Puskás, S., Clemens, B., Emri, M., 2013. BrainCON: Graph theory based multimodal brain connectivity analysis and visualization software, in: *Electronic presentation online system: ECR Congress 2013 (Eds.)*, European Society of Radiology, (S. I.), C-2588.

Stefan, H., Burr, W., Fischel, H., Fröscher, W., Penin, H., 1984. Intensive follow-up monitoring in patients with once daily evening administration of sodium valproate. *Epilepsia* 25, 152-160.

Thatcher, R.W., Biver, C.J., North, D., 2007. Spatial-temporal current source correlations and cortical connectivity. *Clin. EEG Neurosci.* 38, 35-48.

Szava, S., Alan, L., Bosch, J., Clark, I., Jimenez, J.C., 1994. High resolution quantitative EEG analysis. *Brain Topography* 6,3 211-219.

Talairach, J., Tournoux, P., 1988. Co-planar stereotaxic atlas of the human brain: three-dimensional proportional system. G. Thieme, Stuttgart.

Tang, Y., Glauser, T.A., Gilbert, D.L., Hershey, A.D., Privitera, M.D., Ficker, D.M., Szaflarski, J.P., Sharp F.R., 2004. Valproic acid blood genomic expression patterns in children with epilepsy a pilot study. *Acta. Neurol. Scand.* 109, 159-168.

Timofeev, I., Steriade, M., 2004. Neocortical seizures: initiation, development and cessation. *Neuroscience* 123, 299-336.

Wang, C.C., Chen, P.S., Hsu, S.J., Lin, C.T., Gean, P.W., 2012. Valproic acid mediates the synaptic excitatory-inhibitory balance through astrocytes – a preliminary study. *Progress in Neuro-Psychopharmacol. & Biological Psychiatry* 37, 111-120.

Wendling, F., Ansari-Asl, K., Bartolomei, F., Senhadji, L., 2009. From EEG signals to brain connectivity: a model-based evaluation of interdependence measures. *J. Neurosci. Methods* 183, 9-18.

Legends to Figures

Fig. 1

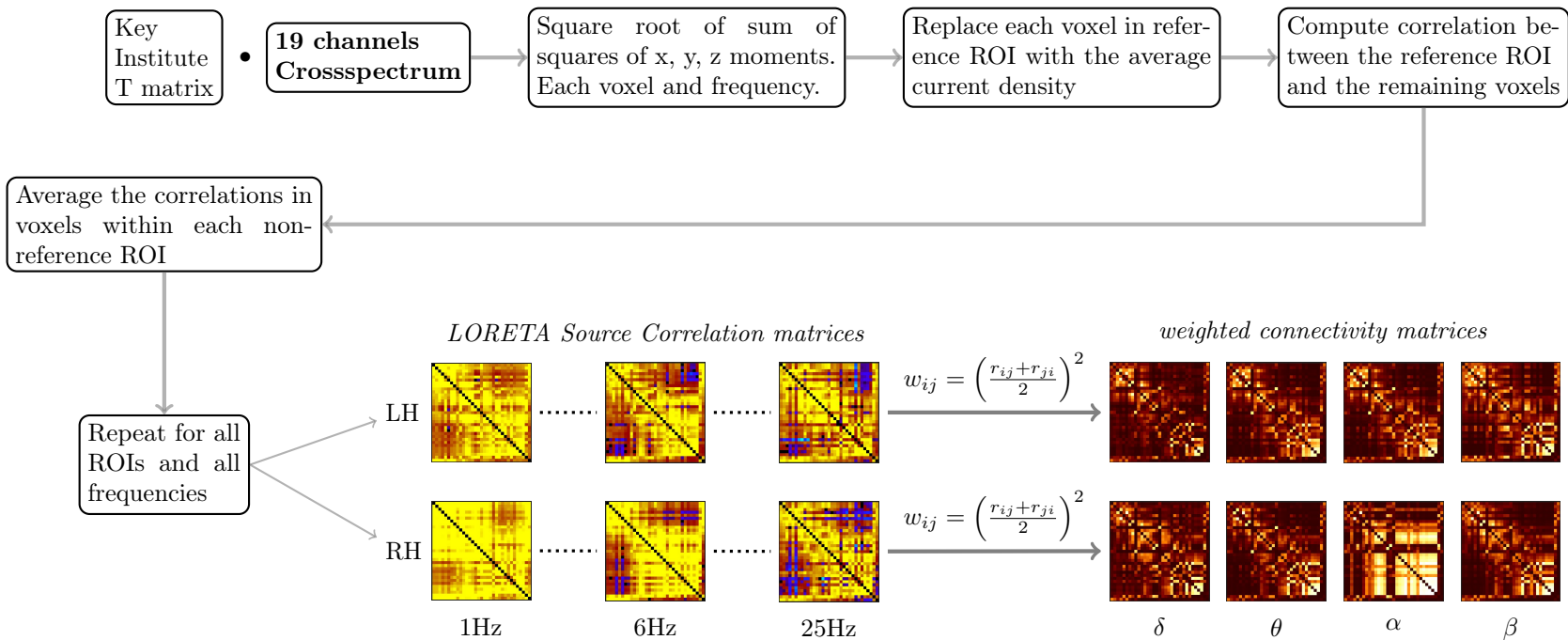
The flowchart describes the steps of the calculation of the LORETA source correlation matrices and the evaluation of the connectivity matrices used in the SPN analysis (left (LH) and right (RH) hemispheres for four broad bands). The LSC flowchart was drawn after Thatcher et al. (2007) with permission of the author.

Fig. 2.

EEGfC group differences in four frequency bands. Top row: untreated IGE group (NAE) versus healthy controls (NC). Middle row: treated condition (VPA) versus untreated condition (NAE) of the IGE group. Bottom row: Treated condition of the IGE group (VPA) versus healthy controls (NC). Colour scale indicates t-values. Only statistically significant ($p < 0.05$, FDR-corrected) differences are demonstrated.

Fig. 3.

EEGfC differences in delta band between the treated IGE group and healthy controls. Middle plot of this figure (top view) shows the same results as the left plot in the bottom row of Fig. 2. Lateral views demonstrate better the lack of statistically significant differences between frontal ROIs in the left and right hemispheres.

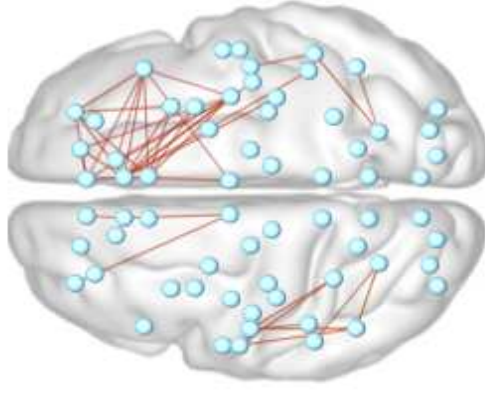
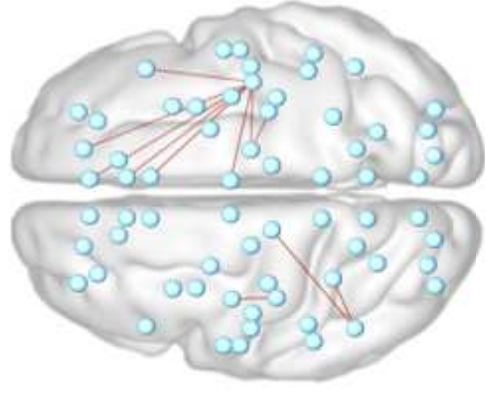
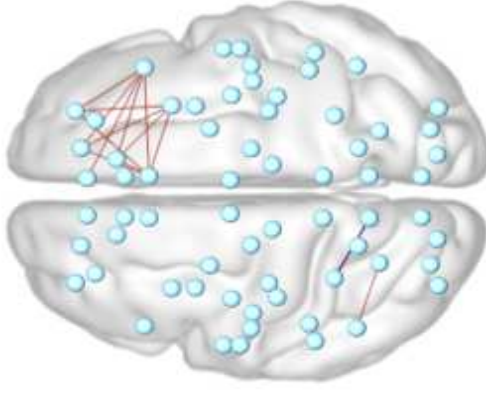


6.0

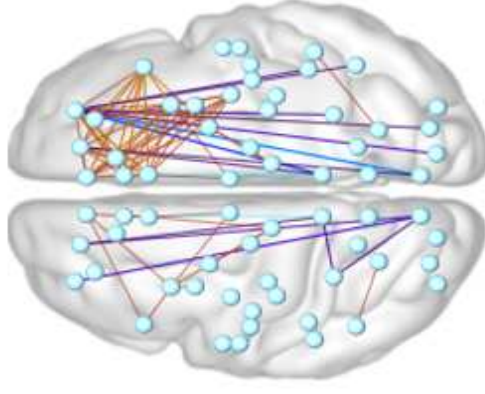
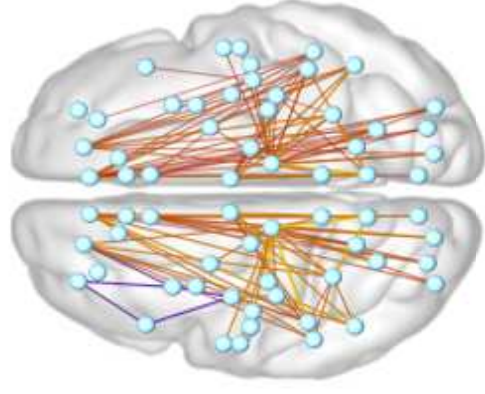
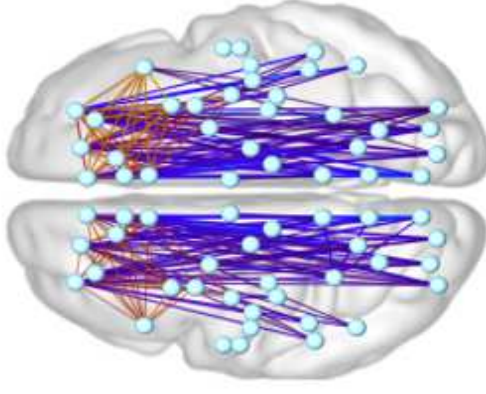
2.7

-2.7

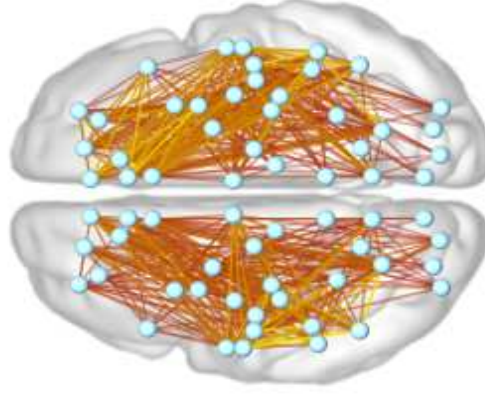
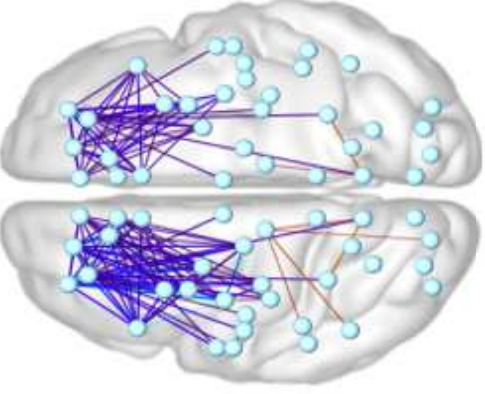
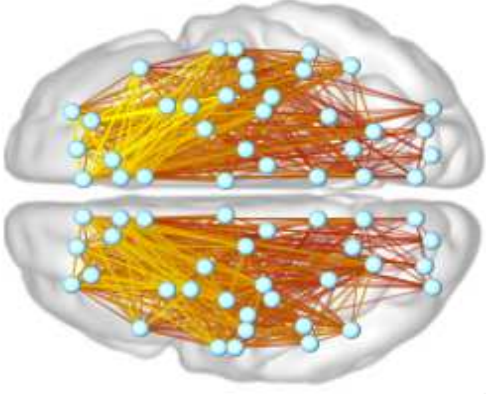
-5.7



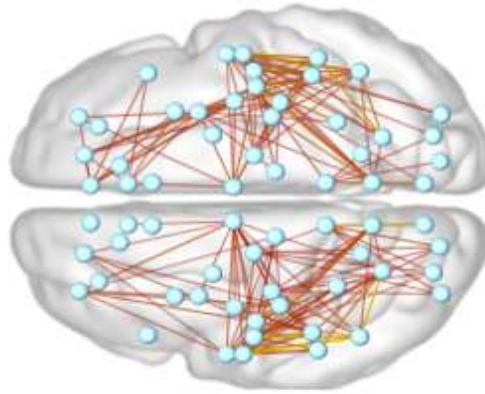
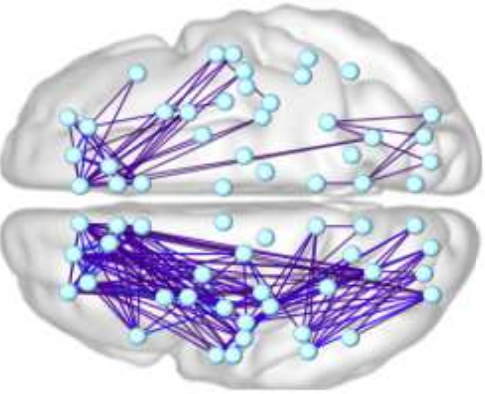
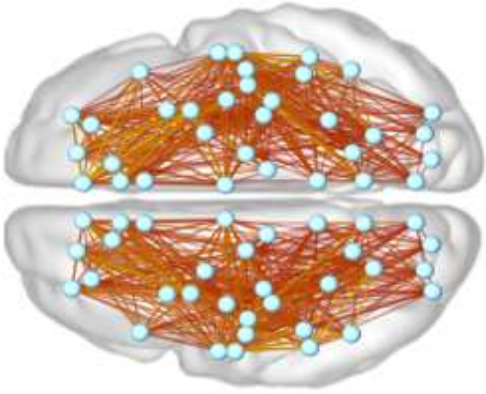
Beta



Alpha



Theta



Delta

NAE
vs.
NC

VPA
vs.
NAE

VPA
vs.
NC

