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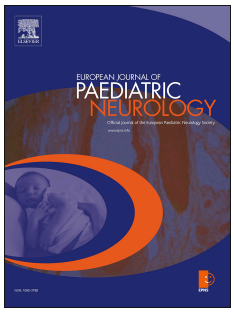

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Multifocal epilepsy in children is associated with increased long-distance functional connectivity: an explorative EEG-fMRI study

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Running Title: Multifocal epilepsy and connectivity

Abbreviations: BECTS = benign epilepsy with centro-temporal spike; BOLD = blood oxygenation level dependency; LGS = Lennox-Gastaut syndrome; FC = functional connectivity; IED = interictal epileptiform discharges; ROI = region of interest; HRF = hemodynamic response function, DMN = default mode network.

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Key words: functional connectivity, children, default-mode network, EEG-fMRI, multifocal epilepsy

ABSTRACT

Objective: Multifocal epileptic activity is an unfavourable feature of a number of epileptic syndromes (Lennox-Gastaut syndrome, West syndrome, severe focal epilepsies) which suggests an overall vulnerability of the brain to pathological synchronization. However, the mechanisms of multifocal activity are insufficiently understood. This explorative study investigates whether pathological connectivity within brain areas of the default mode network as well as thalamus, brainstem and retrosplenial cortex may predispose individuals to multifocal epileptic activity.

Methods: 33 children suffering from multifocal and monofocal (control group) epilepsies were investigated using EEG-fMRI recordings during sleep. The blood oxygenated level dependent (BOLD) signal of 15 regions of interest was extracted and temporally correlated (resting-state functional connectivity).

Results: Patients with monofocal epilepsies were characterized by strong correlations between the corresponding interhemispheric homotopic regions. This pattern of correlations with pronounced short-distance and weak long-distance functional connectivity resembles the connectivity pattern described for healthy children. Patients with multifocal epileptic activity, however, demonstrated significantly stronger correlations between a large number of regions of the default mode network as well as thalamus and brainstem, with a significant increase in long-distance connectivity compared to children with monofocal epileptic activity. In the group of patients with multifocal epilepsies there were no differences in functional connectivity between patients with or without Lennox-Gastaut syndrome.

Conclusion: This explorative study shows that multifocal activity is associated with generally increased long-distance functional connectivity in the brain. It can be suggested that this pronounced connectivity may represent either a risk to pathological over-synchronization or a consequence of the multifocal epileptic activity.

1. Introduction

Temporal correlations of spontaneous low-frequency blood oxygenation level-dependent (BOLD) signal fluctuations exhibit a complex and non-random organization reflecting the co-ordination of different functional systems of the human brain.[1,2] Statistical covariance (i.e. functional connectivity) between resting-state BOLD signals is known to reflect global and local changes the synchronization of neural oscillations.[3,4,5] Therefore it is likely of neuro-physiological origin. Because resting-state BOLD functional connectivity allows investigation of whole brain, large-scale interactions without need for engagement of subjects in task paradigms, it is increasingly being used to identify alterations of functional connectivity due to different pathological conditions.[6,7]

In epilepsy, studies on functional connectivity (FC) gain in importance because ictal as well as interictal epileptiform discharges (IED) result from abnormal inter-neuronal synchronization, which arises locally or in complex networks of temporally interconnected distant brain regions.[8,9] On the one hand, in susceptible individuals it may be possible that abnormal FC at rest may result in abnormal synchronization. Indeed, increased FC has been described in conditions which are associated with increased brain synchronization and recruitment of neuronal bypass such as alpha rhythm, epileptic seizures, neuroleptic medication, drug withdrawal and brain injury.[10,11,12] In conditions with reduced synchronization, such as sedation or anaesthesia, reduced FC has been observed.[13] On the other hand, functional connectivity in brain regions of the default mode network (DMN) is a necessary prerequisite for effective neuropsychological functioning and cognitive abilities.[14] Disturbed connectivity in this network may be associated with cognitive deficits, which have been observed in patients with epilepsy.[15] And, finally, neuropsychological functions such as attention, memory, and executive functions are related to complex networks of interconnected brain structures (for a review see [14]). Epileptic activity originating from any structure in these networks may disturb connectivity and thusly cause cognitive deficits.[16]

There is increasing evidence that patients suffering from epilepsy are characterized by abnormal FC at rest. In patients with idiopathic generalized epilepsies (IGE) the results on FC are inconsistent. Wang et al.[17] found increased FC in the DMN in patients with generalized tonic-clonic seizures, and Bai et al.[18] discovered enhanced between-hemisphere connectivity in childhood absence epilepsy, which in both cases was interpreted as a representation of an abnormally enhanced synchrony. Luo et al.[19] demonstrated decreased connectivity in the same brain structures of patients with absence seizures, and Moeller et al.[20] indicated that connectivity was normal in a group of IGE patients mostly consisting of subjects with juvenile myoclonic epilepsy whereas Luo and colleagues found increased FC of the basal ganglia network in IGE patients.[21] In focal epilepsies the results are more consistent and show decreased FC at rest in both DMN and networks of different cognitive functions.[22,23,24,25,26,27,28] However, there are some few studies which have demonsrated an increased FC in focal epilepsies.[29,30,31]

Here, we compare short-distance (within one and between neighboring Brodmann areas) and long-distance (between remote Brodmann areas) FC in the DMN and in brain regions which are often involved in generation and modulation of epileptic activity between patients with multifocal (Lennox-Gastaut syndrome and multifocal activity in other focal epielpsies) and monofocal epilepsies (benign epilepsy with centro-temporal spikes [BECTS] and focal epilepsies of unknown cause). It is still unclear why some patients present with only one stable focus of epileptic activity and others tend to develop many different changing foci. A machanism associated with increased inter-regional synchronization in multifocal epilepsies has been suggested[32] but has not yet been demonstrated. Moreover, patients with multifocal epileptic activity more frequently present neuropsychological deficits and abnormal cognitive abilities.[33,34,35] Therefore, abnormal connectivity in the DMN may be suggested as a functional substrate for the epileptic encephalopathy in these patients. Finally, syndrome-specific alterations in functional connectivity may also be assumed, since different epileptic

syndromes are characterized by activation of specific neuronal networks during epileptic activity.[36,37,38,39] Thus, in one patient suffering from Lennox-Gastaut syndrome a FC analysis has been performed pre- and postoperatively showing an increase of connectivity in the DMN when multifical IEDs were present at the clinical routine EEG.[40] Therefore, we can hypothesize that functional connectivity in patients with Lennox-Gastaut syndrome could differ from other forms of multifocal epilepsies.

2. Materials and methods

2.1. Subjects

From a database of 247 patients who underwent simultaneous EEG and fMRI recordings in the Department of Neuropediatrics of the University of Kiel since 2005, 33 children fulfilled the criteria of 1) monofocal or multifocal idiopathic or cryptogenic epilepsy 2) with lesions < 1 cm^3 (in order to ensure proper normalization) and 3) being under 18 years of age. The patients were recruited from the Department of Neuropediatrics in Kiel and the Northern German Epilepsy Center Schwentinental-Raisdorf, Germany. The patients were subdivided into two groups according to both the routine EEG and the EEG recorded inside the scanner. All patients with more than one focus in both EEGs were included in the first group called “multifocal” (16 patients, mean age 7.68 +/- 5.06, 5 females). In the “monofocal” group all patients with just one focus in both EEGs were included (17 patients, mean age 9.39 +/- 2.88, 5 females). The groups did not differ significantly with respect to age ($t_{(31)}$ 1.201, p 0.239) or gender ($\chi^2_{(1)}$ 0.013, p 1.000).

In the “multifocal” group, ten patients suffered from multifocal epilepsies of unknown cause and six were classified as multifocal epilepsy of structural-metabolic cause (see Table 1). Nine of these patients had pathological findings in the high-resolution MRI: periventricular leukomalacia (two patients), gliotic or cystic lesions (three patients), delayed myelinization (one patient), global atrophy (one patient), hippocampal malrotation (one patient), and small

tubera (one patient). Thirteen patients were clinically diagnosed as being mentally retarded. One patient suffered from damage after a shaken baby trauma. In order to search for syndrome-specific functional connectivity networks, this group was subdivided in the subgroup "LGS" (n = 10), in which all patients with Lennox-Gastaut syndrome were included, and the subgroup "non-LGS" (n = 6) which included a mixed group of patients with tuberous sclerosis, Pseudo-Lennox syndrome, and patients with structural-metabolic or unknown causes of multifocal epilepsy. All patients had at least two independent foci (two foci in six patients, three foci in nine patients, and four foci in one patient). The type of IEDs was concordant to the routine EEG recorded prior to the EEG-fMRI measurement except of patient 6, who showed spike-slow-wave paroxysms in the EEG obtained outside the scanner and IEDs of spike type obtained inside the scanner. Note that none of LGS patients presented with epileptic spasms at the time of the study, however, 6 patients with LGS had epileptic spasms in the past.

In the "monofocal" group, ten patients were classified as having focal epilepsy of genetic cause and seven as having focal epilepsy of unknown cause. This group was subdivided in the subgroup "BECTS" (n = 8) with benign epilepsy with centrotemporal spikes. Only in one patient of this subgroup did the 3T-MRI reveal discrete periventricular leukomalacia; in all other patients the MRI was normal. All BECTS patients had just one steady spike focus without migration. In the subgroup "non-BECTS" (n = 9), two patients suffered from Landau-Kleffner syndrome. Three patients had the clinical diagnosis of a learning disability. In two patients the MRI was abnormal, showing periventricular leukomalacia in one patient and gliotic lesion in one other patient. All patients had just one epileptic focus. For further details see Table 1.

Diagnoses were made according to the ILAE 2010 classification scheme.[41] The neurological examination and structural MRI (high-resolution T1-, T2-, FLAIR-T2 and diffusion-weighted

imaging) were performed before inclusion into the study. Routine sleep EEGs under sedation (32 electrodes according to the International 10-20 System, the same sedation as for EEG-fMRI, i.e. oral chloral hydrate 50 mg/kg for routine sleep EEGs and 75 mg/kg for sedation in the scanner) were recorded 1 – 2 days before the EEG-fMRI investigation. All children were sedated 30 minutes before MRI scanning, the EEG-fMRI recordings were performed when the children were asleep. A hospital physician was present throughout the examination.

The study was approved by the Ethics Committee of the Faculty of Medicine, University of Kiel, Germany. All participants and their parents were instructed about the study, and written informed consent according to the Declaration of Helsinki (current version, 1996) on biomedical research involving human subjects (Tokyo amendment) was obtained.

2.2. EEG acquisition and processing:

A MR-compatible 32-channel EEG cap ("BrainCap", Falk-Minow Service, Herrsching-Breitbrunn, Germany; 10-20 system plus FC1, FC2, CP1, FC5, FC6, CP5, CP6, TP9, TP10, FT9, FT10, sintered Ag/AgCl ring electrodes) was employed for the MRI recordings with the reference located between Fz and Cz. Electrode impedance was kept below 7 kΩ. EEG was recorded using the MR-compatible system "BrainAmp-MR" (Brainproducts Co., Munich, Germany). Data were transmitted from the high-input impedance amplifier (250-Hz low-pass filter, 10-s time constant, 16-bit resolution, dynamic range of 16.38 mV) which was placed directly behind the head coil inside the scanner room and connected to a computer located outside the scanner room via a fiber optic cable. Foam pads were used to help secure the EEG leads, minimize motion, and improve patient comfort. The scanner (10-MHz sampling rate) was synchronized with the EEG amplifier (5-kHz sampling rate). Online correction of gradient artifacts based on the averaged artifact subtraction (AAS) algorithm was performed using RecView software (Brainproducts Co., Munich, Germany) and enabled visual inspection of spikes throughout the recording.

The EEG was down-sampled to 250 Hz and filtered at 0.03-75 Hz. Then, the averaged gradient artefact was subtracted from the EEG offline using BrainVision Analyzer (version 2.0.1) software.[42] Ballistocardiogramm artefacts were semi-automatically marked and reduced by algorithms of the BrainVision Analyzer software[43] and Brain Electrical Source Analysis software (BESA, MEGIS software Co., Munich, Germany, see[44]). IEDs were marked manually and independently by two experienced neurophysiologists resulting in a consensus set of IEDs using the BESA software package.

2.3. Functional MRI: data acquisition and pre-processing

BOLD-sensitive MRI recordings were performed in a 3T MR scanner (Philips Achieva, Philips, Best, The Netherlands) with a standard 8-channel SENSE head coil. A single-shot T2*-weighted gradient-echo planar imaging sequence was used (TR = 2250 ms, TE = 45 ms, 30 slices, 64 x 64 matrix, slice thickness = 3.5 mm, FOV = 200 mm, flip angle = 90°). Data of 540 brain volumes were acquired during the 20 min fMRI session. The first five images were discarded to ensure steady-state longitudinal magnetization. An anatomical MRI for superimposition with functional images was acquired using a T1-weighted 3-D multiplanar reconstructed (MP RAGE) sequence (1 mm slice thickness, 208 x 208 matrix, 150 slices, FOV = 208 mm, TE = 3.6 ms, TR = 7.8 ms, flip angle = 8°, NSA =2). In a subsequent step, the fMRI data were corrected for movement using the realignment function of the SPM-5 software package (Welcome Department of Cognitive Neurology, London, United Kingdom, http://www.fil.ion.ucl.ac.uk/spm) and normalized to MNI space (Montreal Neurological Institute, Montreal, QC, Canada). No smoothing was performed in the analysis in order to be able to clearly define regions of interest (ROI).[45,46]

2.4. Functional MRI: extraction of resting-state time series

In each individual, the BOLD signal of 15 regions (8-mm spheres) was extracted (see Table 2). In this way, regions representing the default-mode network (1 – 12) as well as subcortical

structures (because of their relevance to LGS[39]) which could be involved in epileptic pathogeneses, were included.[15,38,39,44,47,48,49,50] The coordinates for the selected regions are taken from Fair et al. (2009)[47] for regions 1 – 12 (see table 2) and from Siniatchkin et al., (2011)[39] for subcortical regions 13 – 15. The connectivity analysis was carried following the method described in Fair et al.[47] The BOLD signal was bandpass filtered at 0.005-0.08 Hz (butterworth; filter order: 13; attenuation of stopband: 30 dB) to reduce physiological noise such as cardiac and respiratory artefacts as described by Nomura et al. 2010. Additionally, the IEDs were convolved with a canonical hemodynamic response function (HRF) and regressed out together with the signal from white-matter, cerebrospinal fluid, and movement (realignment parameters from SPM5). With the extracted time series of BOLD signals, a 15x15 square cross correlation matrix was created for each individual, encoding the pair-wise connectivity between al 15 ROIs. In order to take inter-individual differences into account, data of each ROI were Fisher transformed to z-scores. The Schmidt-Hunter method for multiple comparisons was used.[47,51] The direct group comparisons between the main groups and subgroups were performed using two sample two tailed *t*-tests ($p < 0.05$, corrected for multiple comparisons).

Because a consensus for selecting an optimal threshold for the visualization of functional connectivity strength is missing, various thresholds have been used in the literature.[52,53,54,55] In order to reduce the chance of over-estimating the influence of physiologically insignificant and noisy correlations by selecting a low threshold, Stevens and colleagues[54] have have specified a threshold of $r \geq 0.25$ up to $r \geq 0.30$ as significant. Therefore in this study, which is comparable in the number of analysed cases, the results were reported using a threshold for visualization of $r \geq 0.30$ (Figure 1).

2.5.Variance testing

The decomposition of the variance of the data into components explained by the regressors (regressors for realignment parameters, BOLD signal of white matter and cerebrospinal fluid

as well as for interictal epileptiform discharges) was analyzed as follows. For each patient and each ROI a vector was formed from the variances of all regressors (weighted by their corresponding regression coefficients) and from the variance of the residuals of the corresponding regression. The variances of the regressors were then normalized by the sum of the elements of this vector. Finally, the results were averaged over ROIs and grouped into monofocal and multifocal patients. These two distributions were compared by standard *t*-tests. Additionally, the groups were tested with respect to the maximum translation and rotation parameters of each patient based on the realignment parameters of the SPM analysis. The distinction between both groups was tested using standard *t*-tests ($p < 0.05$ was taken for significance).

2.6 Electrophysiology

Different stages of sleep are characterized by distinct patterns of local and global functional connectivity.[56,57,58,59,60] In order to exclude the influence of sleep on results of this study, sleep stages were specified in each patient according to the criteria of Rechtschaffen and Kales.[61] The group differences were then evaluated by the analysis of variance (ANOVA) for the main groups (multifocal vs. monofocal epilepsies) as well as for the subgroups (Lennox-Gastaut syndrome vs. other multofical epilepsies, BECTS vs. cryptogenic focal epilepsies).

3. Results

3.1. Patients with monofocal epilepsies

Patients with monofocal epilepsies were characterized by strong correlations between homologous interhemispheric brain regions. Long-distance functional connectivity, especially in the frontal-occipital direction, was sparse (Figure 1). The strongest interhemispheric correlation was found between the thalami, followed by parahippocampi, lateral parietal cortex, and superior frontal cortex. The subgroup “BECTS” showed similar results. Differences between “BECTS” and the rest of the “monofocal” group were detected for the following correlations (*p*

< 0.05, corrected): left parahippocampus and posterior cingulate cortex, ventral medial prefrontal cortex and brainstem, left thalamus and right inferior temporal lobe, left parahippocampus and right inferior temporal lobe, left inferior temporal lobe and anterior medial prefrontal cortex as well as right inferior temporal lobe and right parahippocampus. These correlations were more pronounced in the "BECTS" group.

3.2. Patients with multifocal epilepsies

Patients with multifocal epilepsies revealed both short- and long-distance functional connectivity. The strongest correlations were found between homologous interhemispheric brain regions such as thalami, superior frontal cortices and lateral parietal cortices. The strongest intrahemispheric correlations could be detected between posterior cingulate cortex and retrosplenial area, posterior cingulate cortex and parietal cortices, posterior cingulate cortex and left superior frontal cortex as well as between the anterior and ventral medial prefrontal cortex, the anterior medial prefrontal cortex and left superior frontal cortex. Moreover, strong correlations were observed between the left temporal lobe and left parietal cortex. Unlike in the "monofocal" group, more correlations were above the correlation coefficient (r) of 0.3. Except for the brainstem, every seed region was functionally integrated.

The subgroup "LGS" was characterized by a pattern of functional connectivity which resembled the functional connectivity of the entire "multifocal" group. Significant differences between "LGS" and the rest of the "multifocal" group were found for the correlations between the ventral medial prefrontal cortex and retrosplenial as well as the right thalamus and brainstem ($p < 0.05$, corrected). These correlations were higher in the "LGS" group.

3.3. Group differences between "monofocal" and "multifocal"

Significant differences with respect to the strength of correlations between patients with monofocal and multifocal epilepsies were found for the following correlations (p <0.05, cor-

rected): between left temporal lobe and ventral medial prefrontal cortex, retrosplenium, left superior frontal cortex, anterior medial prefrontal cortex, right and left thalamus, right and left parahippocampus, right and left lateral parietal cortex and posterior cingulate cortex as well as between left superior cortex, anterior and ventral medial prefrontal cortex, right superior frontal cortex and right and left thalamus. Moreover significant differences were found between anterior medial prefrontal cortex and retrosplenial, anterior medial prefrontal cortex and posterior cingulate cortex, as well as right inferior temporal lobe and right parahippocampal gyrus.

3.4. Variance

Figure 2 shows the percentage of variance in both "multifocal" and "monofocal" groups. Except for IED ($t_{(31)}$ 4.391, $p < 0.001$), the variances for BOLD signal of white matter and cerebrospinal fluid, movement, and residual variance did not differ significantly between both groups (Figure 2). The least pronounced variance was attributed to IED; the most pronounced, to the residual variance which presented the substrate for the analysis performed in this study. The "multifocal" and "monofocal" groups did not differ significantly with regards to the maximum translation (mean 0.64 mm +/- 0.81 mm and 1.44 mm +/- 2.12 mm respectively, $t_{31} = 1.409$, $p = 0.169$) and maximum rotation (mean 0.78° +/- 1.40° and 1.59° +/- 2.26° respectively, $t_{(31)} = 1.228$, $p = 0.229$) parameters based on the realignment parameter of SPM5.

3.5 Differences between the groups concerning electrophysiology and clinical characteristics

Using ANOVA the group differences were tested regarding the sleep stages, duration of epilepsy and seizure frequency. For both main groups (multifocal versus monofocal) sleep stage 2 was present in most of the cases ($F_{(1,31)} = 94.806$; $p < .001$). However the sleep stages did not differ between both main groups ($F_{(1,31)} = .716$; $p = .404$). The same was true for the subgroups ($F_{(3,29)} = .787$; p = .511).

Concerning duration of epilepsy, although the "multifocal" group was characterized by a longer duration (45.0 ± 49.7 months) than the "monofocal" group (31.9 ± 35.16), this difference was non-significant ($F_{(1,31)}$ = .773; p = .386). The most pronounced duration demontrated patients with LGS (51.1 ± 53.54 months). Despite of clear differences in duration, all comparisons between subgroups did not reveal any significant results.

The analysis of seizure frequency demonstrated that patients the "multifocal" group present with higher frequency (17.38 ± 22.61 seizures/week) than patients from the "monofocal" group (3.18 ± 6.7 seizures/week). This difference was significant ($F_{(1,31)}$ = 3.367; p = .032). The high seizure frequency in the "multifocal" group can be attributed to frequent seizures in the subgroup of LGS patients (23.3 ± 26.62 seizures/week): the "LGS" subgroup differed significantly from subgroups of "monofocal" patients ($p < 0.05$) as well as the subgroup of "Non-LGS" patients ($p = 0.042$).

4. Discussion

This explorative study revealed the following two main findings: (1) patients with monofocal epilepsies were characterized by short-distance interhemispheric functional connectivity and patients with multifocal epilepsies demonstrated both short- and long-distance functional connectivity and presented significantly more correlations within the default mode network than patients with monofocal epilepsies; (2) within subgroups, the differences between patients with and without BECTS and with and without LGS were weak and negligible. The following discussion will try to explain these findings, although the presented concept should be treated with caution because of small sample sizes in the investigated groups. The results surely need replication in larger cohorts of patients with monofocal and multifocal epileptic activity.

4.1. Functional connectivity in patients with monofocal epilepsy

Patients with monofocal epileptic activity were characterized by pronounced short-distance functional connectivity, especially between homologous interhemispheric brain areas, and lacked long-distance connectivity, especially between anterior and posterior brain regions. This pattern of resting state functional connectivity resembles those described in the study of Fair et al.[47] which demonstrated rather sparse connectivity in the default-mode network − especially between spatially distant areas − in healthy children during wakefulness compared to adults. Compared to adults, children showed less pronounced functional integration of the brain. Even if the methodology as well as age and gender of the sample from our study are consistent with those from the study of Fair et al., we have to be cautious when drawing conclusions from the comparison of both studies. In our study, children with epilepsy were investigated asleep under sedation whereas in the study by Fair and colleagues children were investigated during wakefulness. Some studies have shown that both sleep and sedation may reduce spontaneous activity in the DMN and disturb functional connectivity between key nodes of this network.[56,57,60] Since particularly the long-distance connections between anterior (frontal) and posterior (parietal and precuneus) brain areas may be affected by sedation and sleep, these factors might have influenced the connectivity pattern observed in our sample. However, we assume that the influence of sleep might only have a moderate influence on the results for the following reasons: In this study, the sedation was not deep (chloral hydrate 75 mg/kg). All patients were sedated and none of the patients reached a higher sleep stage than the 2nd stage of sleep for most of the recording time (Table 1). Furthermore both groups did not differ significantly regarding the sleep stages. There is evidence that superficial non-REM sleep (1st and 2nd stages of sleep) as well as light sedation do not influence connectivity of the default mode network.[62,63,64,65] Even in anesthetized monkeys, the DMN can be observed as a functional unit.[66] It is possible that the low long-distance anterior-posterior functional connectivity observed in our sample may be related to a low structural integrity of the DMN in children.[47,67] Although the influence of sleep and sedation on function-

al connectivity cannot be completely excluded, it is noteworthy that the pattern of functional connectivity which has been described for children during wakefulness[47] is preserved in a non-deep non-REM sleep.

Even if a control group of sedated healthy children is missing (from the ethical point of view), it seems likely that the group of patients with monofocal epilepsies may represent an adequate control group for studies of functional connectivity in children with epileptic encephalopathy as well. The patients from the group with monofocal epilepsies did not differ significantly from the group with multifocal epileptic activity depending on the state of vigilance. Moreover, the majority of children with monofocal epilepsy (except for two patients with Landau-Kleffner syndrome and one patient with partial epilepsy of unknown cause) presented normal psychomotor and cognitive development. The almost normal development allows comparison of functional connectivity patterns in these children with the patterns described by Fair et al.[44] in normally developed children of the same age. And, finally, the normal development may explain discrepancies with other studies which revealed decreased functional connectivity in the DMN in patients with partial epilepsies.[22,23,24,25,26,27,28] These studies investigated adults suffering from mesial temporal lobe epilepsy (mTLE) and frontal lobe epilepsy (FLE). In our study, none of the patients had mTLE or FLE. Many studies demonstrate that the majority of patients with mTLE present cognitive deficits (for a review see[68]). The extent of cognitive deficits correlates significantly with the reduction of FC in patients with mTLE.[22,28] IEDs originating in the temporal lobe in mTLE may interrupt activity in the DMN and thereby cause cognitive disability.[69] The relationship between IEDs and cognitive function in extratemporal epilepsies, however, is unclear. Studies investigating children with BECTS and other forms of cryptogenic and symptomatic monofocal extratemporal epilepsies did not reveal any association between IEDs, deactivation, or connectivity in the DMN.[38,70,71,72] It can be suggested that some specific epileptic syndromes and conditions, such as continuous spikes and waves during non-REM sleep[15] and not just focal extratemporal epileptic activity, may interact with

the DMN and affect FC. It seems likely that BECTS and monofocal epilepsies in this study are characterized by normal FC for the given age.

Previous EEG-fMRI investigations of patients suffering from epileptic encephalopathies provided evidence for syndrome or disease-specific networks.[15,38,39,73] Therefore it is possible that patients with a specific form of epilepsy, for example BECTS, could demonstrate a specific pattern of FC. In such a way, the demonsrated differences between monofocal and multifocal groups may be attribited to differences between syndrom-specific patterns of FC, for example between BECTS and LGS. However, although our patients with BECTS presented slightly different connectivity strengths than other patients from the group with monofocal epilepsies, the differences were very modest and not enough to draw conclusions about syndrome-specific connectivity patterns.

4.2. Increased connectivity in multifocal epilepsy

Patients with multifocal epileptic activity showed increased FC in the DMN and additional brain regions analyzed. As in the group of patients with monofocal epilepsies, the strongest correlations were found between homologous interhemispheric brain regions. Additionally, the study revealed substantially increased long-distance FC between anterior and posterior brain regions. Because patients from the group with multifocal epileptic activity were investigated under sedation and during sleep just as the patients in the monofocal group and also presented intellectual disabilities, reduced FC in the DMN in this group could have been hypothesized (see also section above). However, the opposite was the case. This seems rather unexpected, since young age, sedation, sleep, or intellectual disabilities are associated with reduced DMN FC.[13,14,47,56,57] We therefore conclude that the increased FC in our patients cannot be attributed to effects of these factors but might be associated with the multifocal activity itself.

A number of methodological issues might also explain differences in FC between patients with monofocal and multifocal epilepsies. It is unlikely that movements during data acquisition influenced the connectivity, since the contribution of movement regressors to the variance of BOLD signal (see Analysis of variance in Results) did not differ significantly between the groups. Another important factor which could have influenced connectivity was the number of IEDs leading to a synchronized activation of brain regions. Indeed, the groups of patients with monofocal and multifocal epileptic activity differed significantly according to the number of IEDs and the percentage of BOLD variance explained by the IED. This difference represents a limitation of the study. However, IEDs explain only a very small part of the variance of BOLD signal, namely 0.4 percent in the group with monofocal epilepsy and 1.2 percent in the group with multifocal epileptic activity. Additionally, IEDs were modeled and regressed out of the ROI BOLD time series. Therefore, it is difficult to attribute the number of IEDs for the increased FC in the multifocal group, although effects of IED cannot be excluded completely. The possible independency of the increased FC in epilepsies from the interictal epileptic activity has been demonstrated in a number of studies.[85,86] Luo et al. (2016) have shown an increased frontoparietal FC in patients with BECTS compared with healthy children which was independent of epileptic activity but related to the duration of epilepsy.[86] In the study of Iannitti et al. (2016) the effects of IEDs was even removed from the data analysis, but the spatial pattern of networks of the increased FC remained to be unaffected by this procedure.[85] The authors suggested that the increased FC is most likely to be related to ongoing basic pathological activity which is associated with the epleptogenesis and, among other mechansism and not exclusively with generation of IEDs.

Additionally, duration of disease and seizure frequency may be factors which influence differences in FC between the monofocal and multifocal groups. Although the subgroup of LGS patients was characterized by the longest disease duration, all differences between groups of monofocal and multifocal patients as well as between subgroups were not significant. The

lack of significance may be attributed to the great distribution of the disease duration in small samples. Therefore, the effect of disease duration on FC can not be excluded completely and has to be proven in larger samples of patients. In contrast, the difference between monofocal and multifocal groups for seizure frequency was significant, and the shown differences in FC between the groups may represent the epiphenomenon of seizure frequecy. However, the effect of seizure frequency on FC is unlikely. Note that the differences in seizure frequency between both multifocal subgroups LGS and Non-LGS was significant, although there were no differences between these subgroups for FC. And finaly, the group of multifocal epilepsies presented with more lesions in MRI and with more drug-resistent cases than the monofocal group. Lesions may have had an impact on FC results. Unfortunately, the great heterogeneity as well as different extent and localization of lesions did not enable specific analysis of that impact. Whether drug-resistency is associated with the increased FC in the multifocal group, this remains to be investigated in the future. It may be hypothesized that in pharmacoresistent cases the preserved long-lasting epileptic activity may cause permanent connectivity changes as a result of plasticity triggered by pathological activity.[85] In such a way, the increased FC may be a result of long-lasting drug-resistent epilepsy with permanent seizure activity, and can not be only attributed to the multifocality. Whatever the explanation, the interpretation of our results has to be given with caution, especially considering small sample sizes in the groups studied, because of the great number of co-founding factors.

Since age, state of vigilance, frequency of IED, movements, seizure frequency and duration of disease cannot provide a sufficient explanation for increased FC in the group of patients with multifocal epileptic activity, we assume that this increased connectivity represents a pathological state of the brain, predisposing patients to multifocal activity. It can be suggested that the increased FC may be attributed to the increased excitability within neuronal networks and altered balance between excitatory and inhibitory circuits. For example, GABAergic medication with antiepileptic effects such as propofol and benzodiazepines reduce the

FC in the DMN significantly.[13] In contrast, the reduction of the inhibitory drive from GABA-ergic neurons and increase in cortical excitability following cocaine exposure results in increased FC.[74] Furthermore, the reduced inhibitory drive from the subthalamic nucleus in tremor originates from increased synchrony between basal ganglia and the motor cortex, which may be illustrated by increased cortico-subcortical FC.[74,76,77] It can be suggested that the general increase in cortical excitability following the excitatory/inhibitory imbalance may result in increased neuronal synchrony predisposing individuals to epileptic seizures.[78,79] Electrophysiological studies in animal models as well as human studies have shown increased synchronization in the epileptogenic zone during seizures and interictal periods.[78,80] Patients with multifocal epileptic activity with different epileptogenic regions may be characterized by increased synchronization of widespread areas of the brain.[32] Especially in patients with Lennox-Gastaut syndrome, this widespread cortical synchronization may result from abnormal bottom-up influences of the brainstem reticular formation and pathological thalamo-cortical interactions. All patients with Lennox-Gastaut syndrome from this study were investigated using a conventional general linear model analysis of fMRI time series of IEDs which revealed significant activation of brainstem and thalamus and widespread individual activations in different cortical regions in each particular patient.[39] Although non of the LGS patients presented currently with infantile spasms, the most of them had spasms in the past. The presence of spasms even in the history points at the special role of brainstem as a key structure in the pathogenesis of both spasms and LGS.[38,39,84] The widespreasd cortical activation related to generalized epileptic discharges and spike and wave paroxysms was confirmed recently.[73,81] Since the brainstem exerts control over the gating function of the thalamus through its influence on the reticular thalamic nucleus, brainstem activity may lead to diffuse changes of cortical excitability which predispose the neocortex to multifocal epileptic activity.[82,83] These diffuse changes of cortical excitability may result in increased synchronization (manifest as increased FC in BOLD measurements), as shown in this study. Increased functional connectivity was also shown in a case report of a 5 years old boy suffer-

ing from multifocal activity and clinically diagnosed as LGS.[40] After anterior two-thirds corpus callosotomy and reanalyzation of the same patient the authors were able to demonstrate a nearly physiological architecture of the DMN. At the same time the patient showed remission of seizure types and cognitive development resumed as well as in the routine EEG a reduction of pathological activation was represented. Our data supports these findings. However, we can state that increased FC is not specifically related to Lennox-Gastaut syndrome but represents one possible mechanism which is associated with a risk to multifocal activity. Patients with multifocal epileptic activity with and without Lennox-Gastaut syndrome did not differ significantly according to the pattern and strength of correlation between regions of interest. It remains to be investigated whether reticulo-cortical and thalamo-cortical projections also contribute to increased FC in multifocal epilepsies apart from Lennox-Gastaut syndrome. Whatever the mechanism, the increased FC seems to be associated with multifocal activity, although this association has to be validated in other epileptic encephalopathies and syndromes.

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Table 1

Clinical and demographic characterization. The patients are classified according to the classification system of the International League against Epilepsy (ILAE). The sleep stages are classified according to Rechtschaffen and Kales. The subscripted numbers represents the appearance of the sleep stage in percent of recording time. *Abbreviations*: IED = interictal epileptiform discharges. C = central, F = frontal, O = occipital, P = parietal, T = temporal, lt = left, rt = right. S = spike, SSW = spike-slow-wave. CBZ = carbazepine, CLB = clobazam, LEV = levetiracetame, LTG = lamotrigine, OXC = oxcarbacepine, RUF = rufinamide, STM = sulthiame, TPM = topiramate, VGB = vigabatrin, VPA = valproate. G = genetic cause, S = structural-metabolic cause, U = unknown cause. AA = atypical absences, AS = atonic seizures, CPS = complex partial seizures, CS = clonic seizures, MA = myoclonic absences, MS = myoclonic seizures, RS = rolandic seizures, TCS = tonic-clonic seizures, ToS = tonic seizures. ABPE = atypical benign partial epilepsy, BECTS = benign epilepsy with centrotemporal spikes, LGS = Lennox Gastaut syndrome, LKS = Landau-Kleffner syndrome, PE = partial epilepsy, TS = Tuberous sclerosis. PVL = periventricular leukomalacia, HM = hippocampal malrotation, DM = delayed myelination, *- = normal.

Table 2

Regions of interest used for the functional connectivity analysis. The coordinates are listed in MNI space.

Figure 1

Results of the connectivity analysis. The groups are presented in the rows. Correlation coefficients > 0.3 are displayed for the groups. A thicker line means stronger correlation. At the end, the differences of both groups are presented in terms of *p*-values <0.05, corrected. Slices three and four are trimmed for a better visibility on the underlying layers. Each main-group is divided into (1) “BECTS” vs. “non-BECTS” and (2) “LGS” vs. “non-LGS”. The regions of

interest (ROI) which were analysed, are given in the upper panel of the figure. The coordinates of these ROI are presented in the table 2. The coordinates were drived from previous publicatons.[39,47]

Figure 2

Variance of the data before regression analysis (non-cumulative frequencies are given): IEDs (green), movement parameters (blue), non-gray BOLD signal (signal of cerebrospinal fluid and white matter; red) and the residual variance in gray.

7. References

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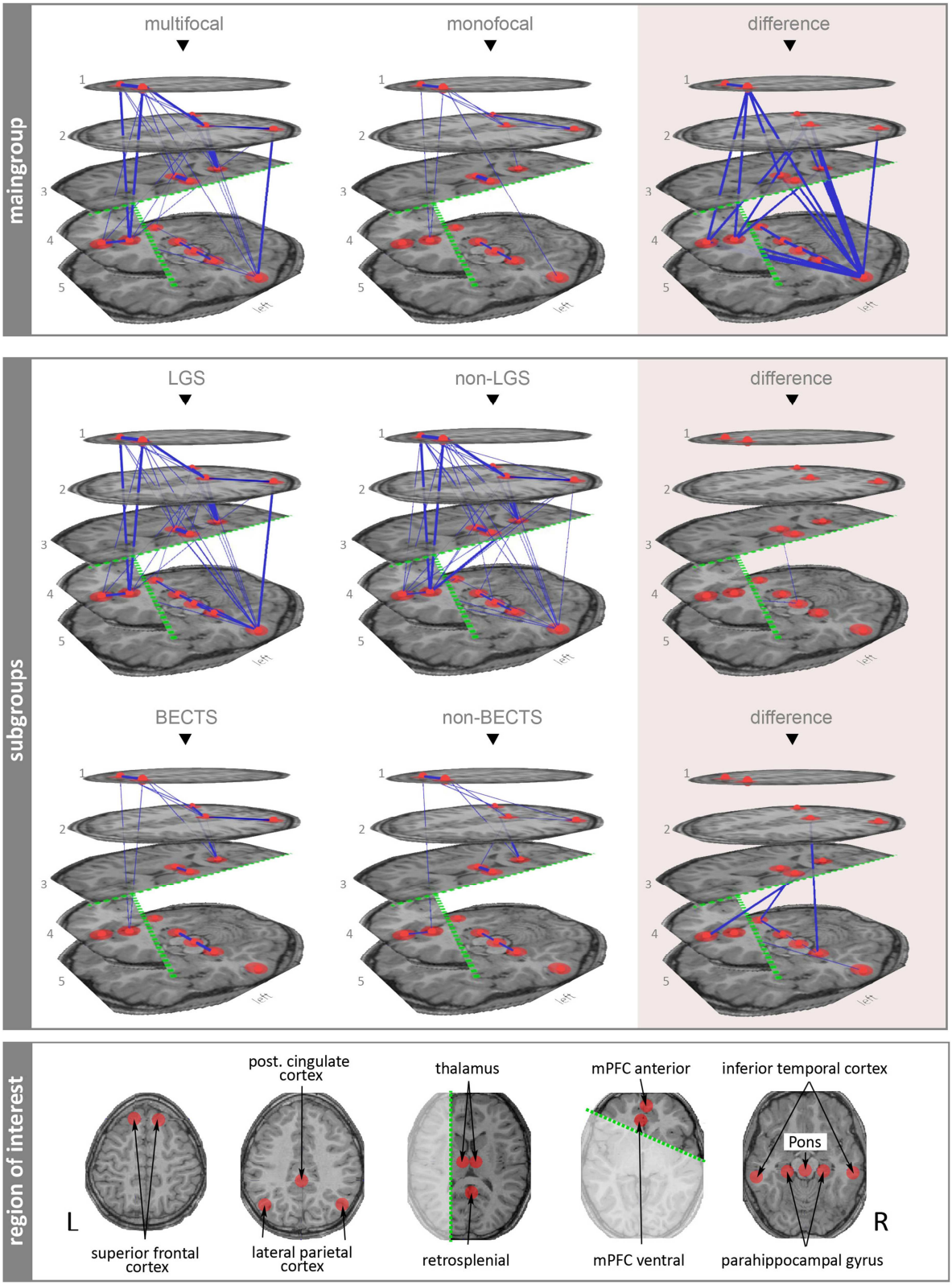
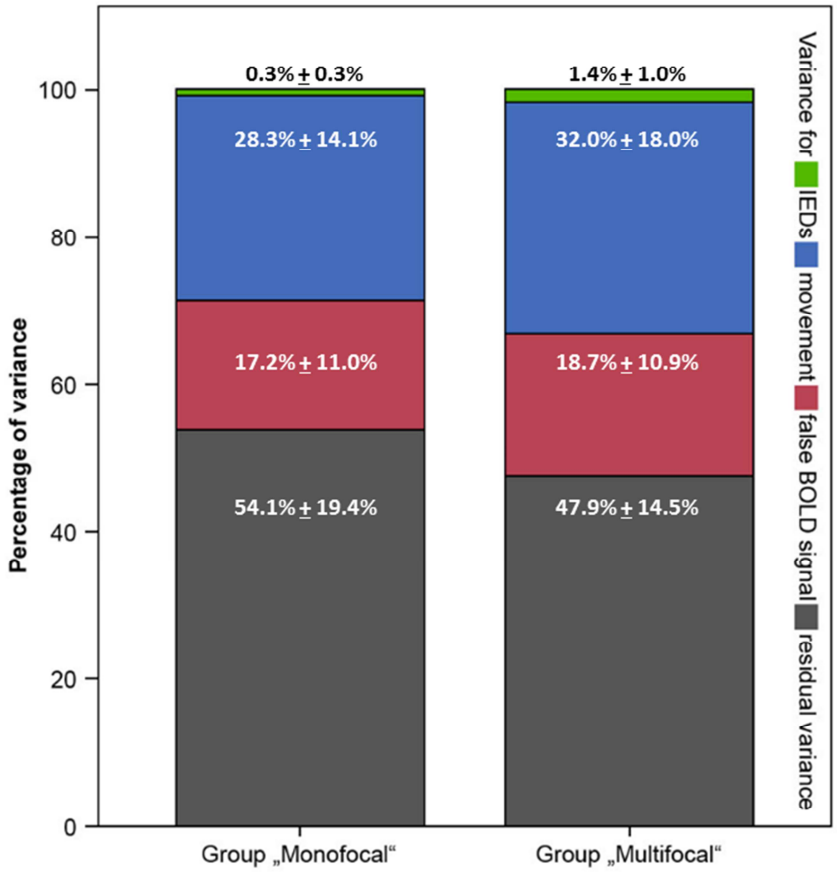
Group		Patient	Sex/Age	Duration of epilepsy (m)	IED focus	Number of IEDs during fMRI	AED	Seizure type	Seizure frequency	Diagnosis	MRI
multifocal	*LGS*	01	F / 6	25	FCT rt, PC lt	1407	LTG, steroid	ToS, MS, AA	5 / day	U / LGS [a]	- *
		02	M / 12	112	F rt, F lt, T rt	1353	RUF, OXC	ToS, AS, CPS	1 / day	U / LGS [a]	-
		03	M / 3	8	T lt, O rt	57	LEV, LTG	CPS, ToS, AS, MA	10 / day	U / LGS [a]	DM
		04	F / 2	10	T lt, T rt, F rt	2603	STM, TPM, VGB	CPS, MS	1 / day	S / LGS [a]	global atrophy
		05	M / 7	37	FT lt, PO rt	153	STM	CPS	2 / day	U / LGS	-
		06	M / 15	122	FT rt, CP lt	1334	VPA	ToS, AS, MS, CPS	1 / day	U / LGS [a]	-
		07	M / 16	144	T rt, T lt, F rt, F lt	305	RUF, LTG, TPM	ToS, AS	3 / day	U / LGS [a]	-
		08	M / 2	3	C rt, F rt, F lt	2020	LTG, steroid	ToS, CPS	1 / week	U / LGS [a]	HM
		09	M / 7	38	F lt, F rt, P rt	847	LTG	ToS, CPS	1 / week	S / LGS [a b]	gliotic lesion
		10	F / 4	12	O rt, P lt	861	STM	ToS, MS	10 / day	U / LGS [a]	-
	non-LGS	11	M / 2	6	O rt, P lt, F lt	82	VPA, STM	TCS, AA	1 / week	S / PE [a]	PVL
		12	F / 1	7	O rt, CP rt, FP lt	2160	VGB, OXC	MS, TS, AS	3 / day	S / TS	tubera
		13	M / 8	12	F rt, FC lt, CT rt	1636	STM	MS, TCS, AS	2 / day	U / ABPE	-
		14	M / 14	24	T lt, P lt, F rt	1074	VPA	MS, AA	1 /day	S / PE [a]	gliotic lesion
		15	M / 11	125	F rt, T lt	1629	STM, LTG	CPS, AA	1 / week	U / PE [a]	cystic lesion
		16	F / 6	36	C rt, O lt, T rt	345	OXC, STM	MS, CPS	1 / day	S / PE [a]	PVL
monofocal	*BECTS*	17	F / 12	78	CT rt	8	STM, CLB	RS, MS	1 / week	G / BECTS	-
		18	M / 6	9	C lt	306	drug-free	RS, MS	3 / year	G / BECTS	-
		19	M / 9	1	PT rt	73	drug-free	RS, TCS	1 / year	G / BECTS	-
		20	M / 8	32	C rt	810	CBZ	RS, CPS	1 / week	G / BECTS	-
		21	M / 10	6	CT rt	154	STM	RS, CS	1 / week	G / BECTS	-
		22	M / 5	1	CP lt	113	STM	RS, ToS	1 / week	G / BECTS	-
		23	F / 9	25	T lt	36	drug-free	RS, CS	1 / year	G / BECTS	-
		24	M / 8	42	C rt	700	VPA	RS, CS	1 / week	G / BECTS	PVL
	non-BECTS	25	M / 10	42	CT rt	213	VPA, LTG, STM	TCS	1 / week	G / LKS [c]	-
		26	M / 5	16	CPT rt	210	LEV	TCS	1 / week	G / LKS [c]	-
		27	M / 13	140	T lt	262	LTG, TPM	CPS	1 / week	U / PE	-
		28	M / 6	28	CT rt	774	STM, OXC	CPS	1 / week	U / PE	PVL
		29	F / 12	14	PO lt	39	STM, OXC	CPS	1 / week	U / PE	-
		30	F / 11	60	F lt	446	VPA	CPS	1 / week	U / PE [c]	-
		31	M / 14	15	O rt	43	LTG	ToS, AA	1 / week	U / PE	-
		32	F / 10	33	P lt	283	OXC	CPS, AA	3 / day	U / PE	gliotic lesion
		33	M / 5	1	CP lt	269	drug-free	AS	1 / year	U / PE	-

[a] mental retardation; [b] state after shaken baby trauma; [c] learning disability

No.	Region	Abbreviation	MNI coordinates		
01	Medial prefrontal cortex (ventral)	mPFC_ven	-3	39	-2
02	Medial prefrontal cortex (anterior)	mPFC_ant	1	54	21
03	Posterior cingulate cortex	Post_cin	-2	-36	37
04	Lateral parietal cortex (left)	L_lat_pa	-47	-67	36
05	Lateral parietal cortex (right)	R_lat_pa	53	-67	36
06	Superior frontal cortex (left)	L_sup_fr	-14	38	52
07	Superior frontal cortex (right)	R_sup_fr	17	37	52
08	Inferior temporal lobe (left)	L_inf_te	-61	-33	-15
09	Inferior temporal lobe (right)	R_inf_te	65	-17	-15
10	Parahippocampal gyrus (left)	L_parah	-22	-26	-16
11	Parahippocampal gyrus (right)	R_parah	25	-26	-14
12	Retrosplenial	Retrospl	3	-51	8
13	Thalamus (right)	R_Thalam	8	-11	12
14	Thalamus (left)	L_Thalam	-8	-11	13
15	Brainstem	Brainste	0	-25	-14


maingroup
multifocal
monofocal
difference
subgroups
LGS
non-LGS
difference
BECTS
non-BECTS
difference
region of interest
L
R
superior frontal cortex
post. cingulate cortex
lateral parietal cortex
thalamus
retrosplenial
mPFC anterior
mPFC ventral
inferior temporal cortex
Pons
parahippocampal gyrus



Highlights

- The pattern of the resting state functional connectivity in children with monofocal epilepsies resemble thus of the healthy children
- Children with multifocal epilepsies and a higher seizure frequency are characterized by an increased long-distance resting state functional connectivity.
- There is no differences in functional connectivity between patients with or without Lennox-Gastaut syndrome