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Increased electroencephalography connectivity precedes epileptic spasm onset in infants with tuberous sclerosis complex

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Clinical Trial Registration: ClinicalTrials.gov, , https://clinicaltrials.gov/show/NCT01780441; , https://clinicaltrials.gov/show/NCT01767779

Summary

Objective: To identify whether abnormal electroencephalogram (EEG) connectivity is present prior to onset of epileptic spasms (ES) in infants with tuberous sclerosis complex (TSC).

Methods: Scalp EEGs were collected prospectively in infants diagnosed with TSC in the first year of life. This study compared the earliest recorded EEG from infants prior to ES onset (n=16) and from infants who did not develop ES (n=28). Five minutes of stage II or quiet sleep was clipped and filtered into canonical EEG frequency bands. Mutual information values between each pair of EEG channels were compared directly and used as a weighted graph to calculate graph measures of global efficiency, characteristic path length, average clustering coefficient, and modularity.

Results: At the group level, infants who later developed ES had increased EEG connectivity in sleep. They had higher mutual information values between most EEG channels in all frequency bands adjusted for age. Infants who later developed ES had higher global efficiency and average clustering coefficients, shorter characteristic path lengths, and lower modularity across most frequency bands adjusted for age. This suggests that infants who went on to develop ES had increased local and long-range EEG connectivity with less segregation of graph regions into distinct modules.

Significance: This study suggests that increased neural connectivity precedes clinical ES onset in a cohort of infants with TSC. Overconnectivity may reflect progressive pathological network synchronization culminating in generalized ES. Further research is needed before scalp EEG connectivity measures can be used as a potential biomarker of ES risk and treatment response in pre-symptomatic infants with TSC.

Keywords

functional connectivity; graph theory; infantile spasms; mutual information; functional connectivity; graph theory; infantile spasms; mutual information

Introduction

Disrupted early brain development at the cellular level in tuberous sclerosis complex (TSC) causes aberrant neural connections and leads to neurological disorders including epilepsy, autism spectrum disorder, and intellectual disabilities.[1–3] Epilepsy develops in in up to 85% of children with TSC, and both epileptic spasms (ES) and early refractory focal seizures (FS) are associated with a poor neurodevelopmental prognosis.[4–6] For ES specifically, early treatment is likely associated with improved outcomes.[7,8] An open label pilot study suggested that treatment of TSC patients with an abnormal electroencephalogram (EEG) prior to onset of ES may improve neurological outcome.[9]

Infants with TSC are often identified by other disease manifestations prior to developing neurologic disorders.[10–12] Expanding knowledge of the biochemical and neural circuitry disruptions occurring in TSC, as well as advances in methods to non-invasively measure neural connectivity in humans, allows us to better understand the processes leading to

epilepsy. These insights could allow treatment of the underlying causes of epilepsy in an individualized and targeted manner.[13,14]

Epilepsy develops as a result of aberrant neural connections that allow for excessive neuronal discharges to organize and propagate.[15] For ES, many different mechanisms of seizure generation have been postulated. Given the wide range of genetic, metabolic, and structural abnormalities associated with ES, it is possible that multiple mechanisms converge on this one seizure phenotype.[16] Proposed mechanisms include desynchronization or imbalance of cortical and subcortical activity, abnormalities in early brain development and neuronal migration, and abnormal synaptogenesis.[17–20] However, it is also possible that ES have a focal onset with rapid spread throughout the brain.[21] One study showed that infants with West syndrome and hypsarrhythmia had increased long-range EEG coherence in multiple frequency bands in sleep after onset of ES.[22] Another study showed that infants after ES onset had decreased long-range temporal correlation in the beta band of awake EEG. This correlation increased to levels found in normal controls following successful ES treatment.[23] The same cohort had increased broadband cross-correlation connectivity prior to treatment, and those with the highest number of strong connections had the best treatment response.[24] Therefore, EEG connectivity in ES may be state-dependent and vary based on frequency bands and connectivity measures examined. Our group has previously reported on 28 infants with TSC studied prior to epilepsy onset. We found that of 19 infants who developed epilepsy, 14 (74%) had epileptiform discharges found on EEG prior to onset of clinical seizures.[25] These data suggest that abnormal brain activity on EEG may be seen before onset of clinical epilepsy in infants with TSC.

Brain networks can be described by topological analysis of EEG connectivity metrics. In addition, graph measures characterize the architecture of the network and are well-suited to the study of epilepsy.[26,27] We hypothesized that abnormal neural connectivity in TSC underlies ES development and can be quantified with network measures of scalp EEG data. In a multicenter prospective observational study of serial EEG in infants with TSC, we compared EEG connectivity before ES onset to EEG connectivity in infants who did not develop ES.

Methods

Study design, subject, and EEG characteristics

Infants newly diagnosed with TSC by clinical or genetic testing criteria were enrolled in two prospective, observational, multi-center studies of biomarkers for epilepsy and autism spectrum disorders in TSC (“Potential EEG Biomarkers and Antiepileptogenic Strategies for Epilepsy in TSC” [] and “Early Biomarkers of Autism Spectrum Disorders in Infants with Tuberous Sclerosis Complex” []). The studies collected clinical and genetic data, neuropsychological testing, serial MRI, and serial EEGs, through age three years. Complete study protocol and design was reported previously.[12] The study protocols were approved by the Internal Review Board at each site with direction from the leading regulatory core at Cincinnati Children’s Hospital Medical Center. Informed consent was obtained from the parents or legal guardians of all study participants. The analysis reported here used one EEG per subject recorded prior to onset of ES (Pre-ES, n=16) or from subjects who did not

develop ES (No-ES, n=28). For each subject, the single earliest analyzable EEG with at least five minutes of identifiable stage II or quiet sleep was included in the current study. EEGs were acquired uniformly across study sites, with electrodes placed according to the 10–20 international placement system, at a sampling rate of 2000 Hz, as previously described.[25] Study EEGs were read by two board-certified clinical neurophysiologists who recorded findings according to the National Institute of Neurological Disorders and Stroke Common Data Elements (http://www.commondataelements.ninds.nih.gov/) for scalp EEG.[28] Discrepancies between readers were adjudicated by a third board-certified clinical neurophysiologist. Data elements included in this study were presence of interictal epileptiform activity and whether it was generalized or localized, presence of slowing, presence of seizures, and presence of hypsarrhythmia.

See Table 1 for demographics and group comparisons. There was no significant difference between the groups by age at EEG (Wilcoxon rank sum test p=.34) or sex (*chi-square* test p=.32). There were no infants with *TSC1* variants or no mutation identified in the Pre-ES group (Fisher's exact test p=.01). There was no significant difference between the groups in the proportion of infants who had FS with onset either before or after the EEG (*chi-square* test p=.30), age of FS onset (Wilcoxon rank sum test p=.73), or time from EEG to FS onset (Wilcoxon rank sum test p=.29). EEGs in both groups had focal interictal epileptiform activity (*chi-square* test p=.12), a smaller number had slowing (Fisher's exact test p=.65), and prevalence did not differ significantly between groups. No EEGs had generalized interictal epileptiform activity.

Most infants were not on anticonvulsants at the time of EEG recording. Five subjects were on anticonvulsant medications at the time of the EEG. One Pre-ES subject was on vigabatrin and phenobarbital for FS. One No-ES subject was on vigabatrin, clobazam, and phenobarbital for FS. Another No-ES subject was on vigabatrin without any record of clinical seizures. This subject had focal epileptiform abnormalities on EEG, so it is possible that s/he was started on vigabatrin as preventative therapy. One No-ES subject was on oxcarbazepine and another was on levetiracetam for other seizure types (not ES or FS).

EEG processing pipeline

We developed an EEG processing pipeline (Figure 1) to generate measures reflecting functional connectivity during stage II or quiet sleep between cortical regions measured by scalp EEG in canonical EEG frequency bands. We computed between-channel connectivity measures using mutual information (MI), a nonlinear measure of shared information between two signals. MI has several advantages as a measure of EEG connectivity for this study: (1) it is relatively straightforward to compute; (2) it can identify nonlinear and anticorrelated relationships between signals; and (3) it can be used on longer signal recordings without being significantly affected by transient artifacts or epileptiform discharges.[29–31] All data and statistical analyses were performed using custom code and standard or publicly-available toolboxes in MATLAB R2017a (The MathWorks Inc., Natick, MA). Full details of EEG preprocessing, filtering and MI calculation are included in the Methods supplement.

Computation of graph measures based on MI

MI values were used to compute graph measures summarizing different aspects of EEG connectivity for each subject to examine age-related changes and between-group differences. The Brain Connectivity Toolbox was used to compute weighted graph measures of global efficiency, characteristic path length, average clustering coefficient, and modularity.[32] An extensive introduction to graph theoretical measures can be found in previous work from our group.[33] Briefly, global efficiency and characteristic path length are complementary measures of the average shortest path between any two graph nodes, and measure connection strengths between nodes that are not directly connected. Clustering coefficient calculates the strength of connected neighboring nodes for each node, reflecting local connectivity. Modularity reflects the proportion of local connections within modules (sub-graphs) to connections between modules, reflecting the level of segregation in a network.[32]

Group statistical comparisons

To evaluate group differences, we compared MI connectivity values between each pair of channels in each frequency band across Pre-ES and No-ES groups. Because of the skewed distribution of MI, MI values were log transformed using the natural logarithm (log MI) and compared using a two-sided non-parametric Wilcoxon rank sum test (Supplemental figure 1). Graph measures were compared across Pre-ES and No-ES groups using an analysis of covariance (ANCOVA) model with the graph measure as the dependent variable, adjusting for logarithm of age as a covariate. To determine if the presence of epileptiform abnormalities affected connectivity, MI and graph measures from subjects with (n=18) and without (n=26) focal epileptiform abnormalities were compared using the same methods, and the original analysis was also run excluding subjects with epileptiform abnormalities. To correct for multiple comparisons across multiple MI channels and across all graph measures, we applied the Benjamini-Hochberg false discovery rate (FDR) procedure to correct for the expected proportion of false discoveries relative to total discoveries.[34] The q-value for the expected proportion of false discoveries was set at 5% (q=0.05).

Results

Overall, infants with TSC who would later develop ES had increased EEG connectivity, both when quantified between channel pairs using MI and over the entire scalp EEG using graph measures. These results were not due to the presence of focal epileptiform EEG abnormalities.

MI connectivity differences between groups

In examining shared EEG signal between cortical regions at a group level, we found that MI between pairs of EEG channels was higher in infants who went on to develop ES than in those who did not. While both groups had the strongest connections between physically-adjacent channels, there were more moderate-strength connections between more spatially-distant channels in the Pre-ES infants than the No-ES infants (Figure 2A). The difference between the groups became more apparent when we compared age-adjusted MI between each channel pair in the Pre-ES group and the No-ES group. The Pre-ES group had higher MI connections between most spatially-distant channels within and between hemispheres

(Figures 2B and 2C). Smaller MI differences in lower frequency bands were due to the smaller range of absolute MI values in lower frequency bands (see Supplemental figure 1).

Graph measure differences between groups

We used weighted graph measures to quantify the architecture of functional EEG networks. Age-adjusted graph measures in most frequency bands demonstrated significantly higher connectivity in Pre-ES than No-ES groups. Global efficiency was significantly higher in Pre-ES than No-ES subjects in delta, theta, alpha, beta, and high gamma bands (Figure 3A) with significantly shorter characteristic path lengths in delta, theta, alpha, gamma, and high gamma bands (Figure 3B). Pre-ES subjects also had higher average clustering coefficients (Figure 3C) and lower modularity (Figure 3D) in all frequency bands. See Supplemental table 1 for statistical comparison values. Supplemental figure 2 shows the scatter plots of all graph measures versus age with the corresponding estimated fit for each group. While the relatively high inter-individual variability in graph measures led to larger confidence intervals, the estimated differences between groups remained consistent in direction in each measure across all frequency bands. These results demonstrate that MI graphs in the Pre-ES group had stronger local and long-range connectivity with less segregation of graph regions into distinct modules.

Effect of focal epileptiform abnormalities and slowing on MI and graph measures

None of the included EEGs showed hypsarrhythmia. Two infants later had hypsarrhythmia prior to ES onset, and one had hypsarrhythmia at the time of ES onset. Previously reported findings in a subset of this cohort by our group found that epileptiform discharges preceded onset of clinical seizures in 74% of infants but did not find that hypsarrhythmia consistently preceded onset of clinical seizures.[25] In the current study, focal epileptiform EEG abnormalities were more prevalent in the Pre-ES group than the No-ES group, but the difference was not statistically significant (56% vs 32%, *chi-square* test p=.12).

To determine whether focal epileptiform EEG abnormalities contributed to differences in MI between ES groups, we compared age-adjusted MI in subjects whose EEGs did and did not have focal epileptiform abnormalities, analogous to the method used to compare MI in Pre-ES and No-ES subjects. We found no statistically significant differences in MI between subjects with and without focal epileptiform abnormalities in any channel pair in any frequency band. We also found no differences in age-adjusted graph measures between subjects whose EEGs did and did not have focal epileptiform abnormalities (all comparison FDR-corrected p>.05, **see** Supplemental table 2). When subjects with focal epileptiform abnormalities were excluded from the analysis, leaving nine Pre-ES and 17 No-ES subjects to be compared, we found similar group difference estimates in the same direction in all graph measures and frequency bands, as found in the analysis including all subjects. However, the differences no longer reached statistical significance, likely due to a loss of power from the smaller sample size.

For the six EEGs with slowing reported, none were outliers on any graph measure, and there was no statistically significant effect on any graph measure in any frequency band when slowing was added as another predictor to the statistical model.

Relationship of graph measures with age

All graph measures showed increasing connectivity correlated with age in the higher frequency bands (beta, gamma, and high gamma) in both Pre-ES and No-ES groups. Additionally, modularity showed significant decreases correlated with age in theta and alpha frequency bands. Global efficiency and average clustering coefficients positively correlated with age, and characteristic path length and modularity negatively correlated with age. These changes occurred logarithmically, showing larger changes at younger ages and smaller changes at older ages. For example, beta band global efficiency was positively correlated with log age (Figure 4A), characteristic path length was negatively correlated with log age (Figure 4B), average clustering coefficient was positively correlated with log age (Figure 4C), and modularity was negatively correlated with log age (Figure 4D). Excluding the two oldest No-ES subjects from the analysis did not substantially change any group differences, and only the increase in high gamma global efficiency with age was no longer statistically significant. See Supplemental table 1 and Supplemental figure 2 for complete results from all frequency bands and graph measures, and Supplemental figure 3 for results excluding the two oldest No-ES subjects.

Discussion

Our findings indicate the presence of increased EEG connectivity in sleep before ES onset in a cohort of infants with TSC. Between-channel MI connectivity values were consistently higher in the Pre-ES than the No-ES group across all frequency bands. Pre-ES infants also had a greater number of additional connections between spatially-distant channels within and across hemispheres with MI above the 60^{th} percentile. Both groups had the strongest connections between physically-adjacent channels, possibly representing volume conduction of electrical signal. Beta, gamma, and high gamma frequency bands also showed higher anterior-posterior MI values that likely reflected shared longitudinal bipolar channels. These common patterns across groups dropped out when the groups were compared, showing that MI values were higher in the pre-ES group between most spatially-distant channels within and between hemispheres (Figures 2B and 2C).

Slow-wave activity is thought to contribute to long-range synchronization across cortical regions and delta, alpha, and beta activity may reflect thalamocortical connections.[35] Increased connectivity in both long-range cortico-cortical and cortical-subcortical connections may allow for increased synchronization across large cortical regions and facilitate the development of generalized seizures. In TSC, (multi-)focal lesions are thought to cause ES, but the clinical semiology of ES reflects a widespread cerebral involvement, concordant with the generalized EEG pattern seen. Our work suggests that excessive synchronization of brain activity precedes the development ES in TSC. Additionally, the increased EEG connectivity in sleep that was previously found in hypsarrhythmia after onset of ES[22] can also be seen in this study before onset of ES and in the absence of hypsarrhythmia.

Graph measures complement MI connectivity strength measures and allow for assessment of the organization of functional brain networks. At a group level, all graph measures in almost all frequency bands showed that Pre-ES infants had higher connectivity than No-ES infants.

Increased global efficiency and shorter characteristic path lengths likely reflect increased long-range connectivity. Higher average clustering coefficients likely reflect increased short-range connectivity. Lower modularity may indicate that the increase in long-range connectivity is greater than the increase in short-range connectivity.

In a previous study, we found that older children with TSC with and without autism had decreased mean coherence, clustering coefficients, and global efficiency, and longer path lengths when compared to control subjects with and without autism.[33] However, that study did not specifically investigate epilepsy-related differences within the TSC population, and the differences were found in the lower alpha band in awake EEG of older children. In the current study, we found higher MI in all frequency bands in sleep in infants and young children with TSC who developed ES. Additionally, we found that increased MI connectivity was associated with changes reflecting increased connectivity in both long-range and short-range network metrics.

All graph measures demonstrated increasing connectivity with age in beta, gamma, and high gamma frequency bands. Modularity also declined with age in the theta and alpha frequency bands. This correlation was seen in both Pre-ES and No-ES groups, but the higher connectivity seen in the Pre-ES group may indicate a disruption of normal brain development processes, such as developmental immaturity of neuronal inhibition or failure of synaptic pruning. It may also result from a pathological increase in neural connectivity due to excessive activity-dependent plasticity, predisposing to epilepsy.[36,37] Increased EEG connectivity may demonstrate a neurophysiologic correlate of early brain overgrowth seen in infants later diagnosed with autism.[38,39]

Time from the first EEG to ES onset ranged from 24 to 197 days. We did not find a correlation between graph measures and time from EEG to ES onset. This may be due to a lack of power to detect a correlation. However, it may indicate that these graph measures reflect more stable underlying connectivity rather than a changing level of epileptiform EEG activity. This interpretation is also supported by the finding that graph measures did not differ between subjects who did or did not have focal epileptiform EEG abnormalities and no effect of EEG slowing on graph measures was found.

Future work on changes of graph measures with age will need to use multiple EEGs recorded longitudinally on the same subjects to identify changes directly related to age, brain development, seizure onset, and treatment effects and response. Following the cohort of patients enrolled in the study “Early Biomarkers of Autism Spectrum Disorders in Infants with Tuberous Sclerosis Complex” () as they develop to school age would enable us to address this critical question.

This work carries some limitations: First, the Pre-ES group was found to only include infants with pathogenic *TSC2* gene variants, while the No-ES group included infants with *TSC1*, *TSC2*, and no identified pathogenic variants. This is consistent with previous studies showing that ES are more prevalent in individuals with *TSC2* variants.[4,12,40]

Second, despite the multicenter prospective design, we identified only a small number of infants who had EEGs recorded prior to onset of ES. We analyzed the first available EEG in

both groups to ensure that the groups were comparable and that the findings would be clinically relevant. When the analysis was repeated using the last EEG prior to ES onset in the Pre-ES group, we found very similar results indicating higher connectivity in graph measures prior to ES onset (results not shown).

Third, while the clinical semiology of ES seen in TSC appears similar that seen in ES from other structural or genetic causes, they may stem from a different pathophysiological mechanism. Several large studies on the natural history and treatment of ES in infants have been performed, yet the mechanism of ES in human infants remains unclear.[41–44] Thus, these findings should be replicated and expanded in a larger cohort of infants with TSC and others at risk of ES, such as infants with Down Syndrome or perinatal brain injury, who could be followed prospectively with EEG before clinical onset of ES.

Finally, these connectivity measures are not sensitive or specific enough to be used as the sole predictor of the likelihood of a single subject developing ES. However, the reported group differences between infants with TSC who do and do not develop ES could represent pathophysiologic changes during the development of ES. For scientific rigor, reproducibility and transparency, we (1) calculated the most appropriate mutual information parameters based on a standard rule; (2) applied the established parameters consistently; (3) used simple graph measures; and (4) presented all results in all frequency bands. While findings based on quantitative EEG analysis may be sensitive to changes in parameters and use of different measures,[31] it is reassuring that many findings in EEG connectivity are robust to differences in the choice of coupling measure and analysis methods.[33,45] Future work will investigate whether a combination of EEG measures, diffusion-weighted MRI white matter connectivity, and TSC clinical features improves the predictive ability of these measures for use as biomarkers of epilepsy risk and treatment response.

Infants with TSC show evidence of abnormal neural connectivity prior to onset of ES. Once treated successfully for 6–12 months, ES often do not relapse, suggesting a developmental window of vulnerability.[42] Whether this vulnerability is reflected by the network measures and can serve as a marker of therapeutic success should be investigated by comparison with patients with relapsing or refractory ES. Ongoing clinical trials are investigating the risks and benefits of pre-symptomatic treatment with Vigabatrin for epilepsy in infants with TSC (“Preventing Epilepsy Using Vigabatrin In Infants with TSC” [] in the United States; “Long-term, Prospective Study Evaluating Clinical and Molecular Biomarkers of Epileptogenesis in a Genetic Model of Epilepsy – Tuberous Sclerosis Complex” [] in the European Union). In addition, future studies could investigate whether disease-modifying therapy inhibiting the mechanistic target of rapamycin normalizes network connectivity. Network connectivity analyses could advance insight into brain development changes occurring during such early preemptive interventions.

Conclusion

Increased EEG connectivity in infants with TSC precedes clinical ES onset in our study cohort. This developmental overconnectivity involves all frequency bands, both short- and long-range connections, and alters network connectivity architecture. Excess connectivity

may reflect progressive pathological network synchronization culminating in generalized epileptic spasms. Several studies have now found group-level differences in scalp EEG connectivity in infants with ES.[22–24] While some of these measures reflect response to treatment, none are sensitive or specific enough to be used as a predictor of individual clinical outcomes. Scalp EEG connectivity measures warrant further investigation into their use as a potential biomarker of epilepsy risk and treatment response in pre-symptomatic infants with TSC and other infants at risk of developing ES, in combination with other clinical features and physiologic measures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ANCOVA	analysis of covariance
EEG	electroencephalogram
ES	epileptic spasms
FS	focal seizures
FDR	false discovery rate
MI	mutual information
TSC	tuberous sclerosis complex

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Key Points

- Infants with tuberous sclerosis have increased mutual information connectivity in sleep on scalp EEG before epileptic spasm onset.
- Graph measures demonstrate increases in long-range and short-range EEG connectivity prior to epileptic spasm onset.
- EEG mutual information and graph measures in higher frequency bands showed increases in connectivity correlated with age.

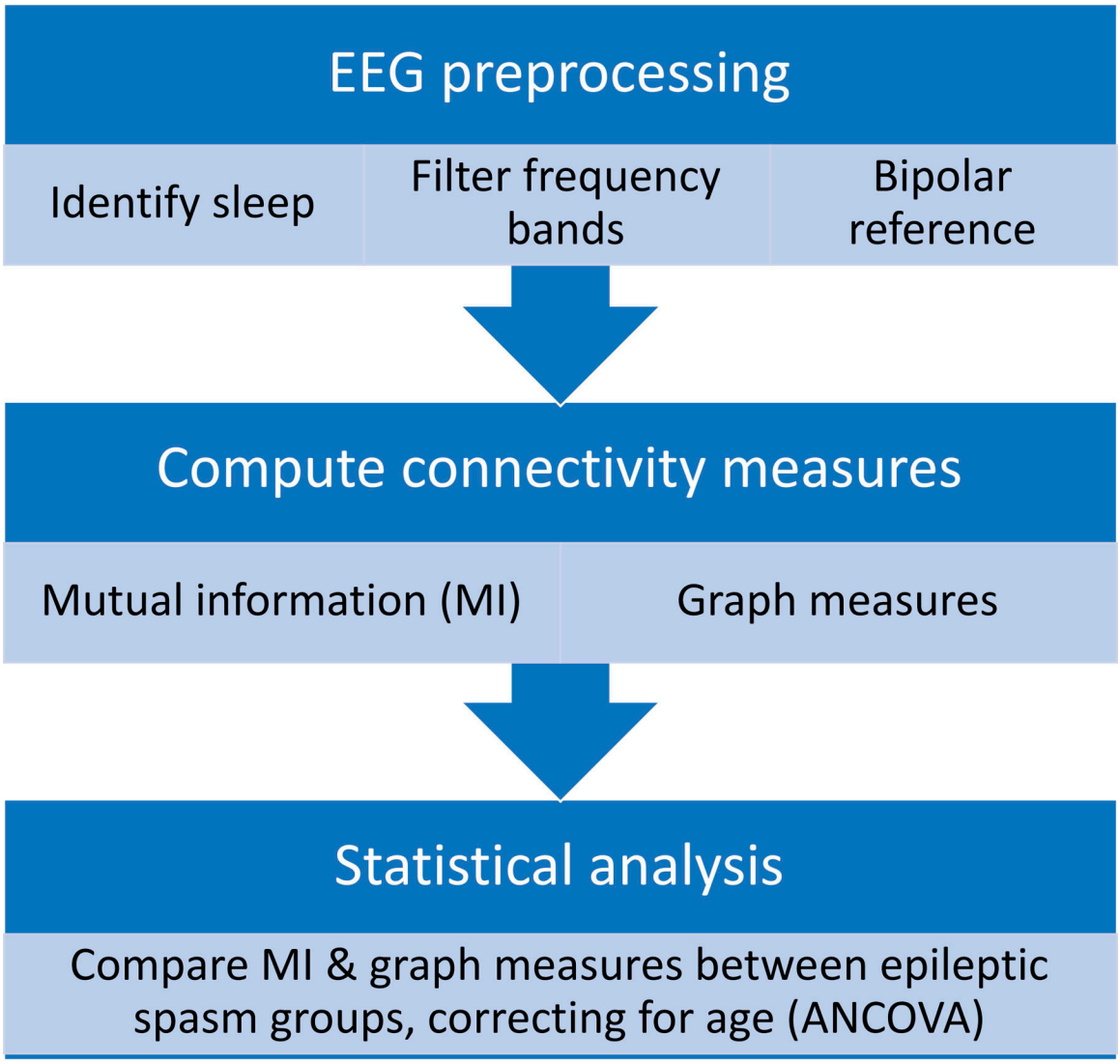

Figure 1.
EEG processing pipeline

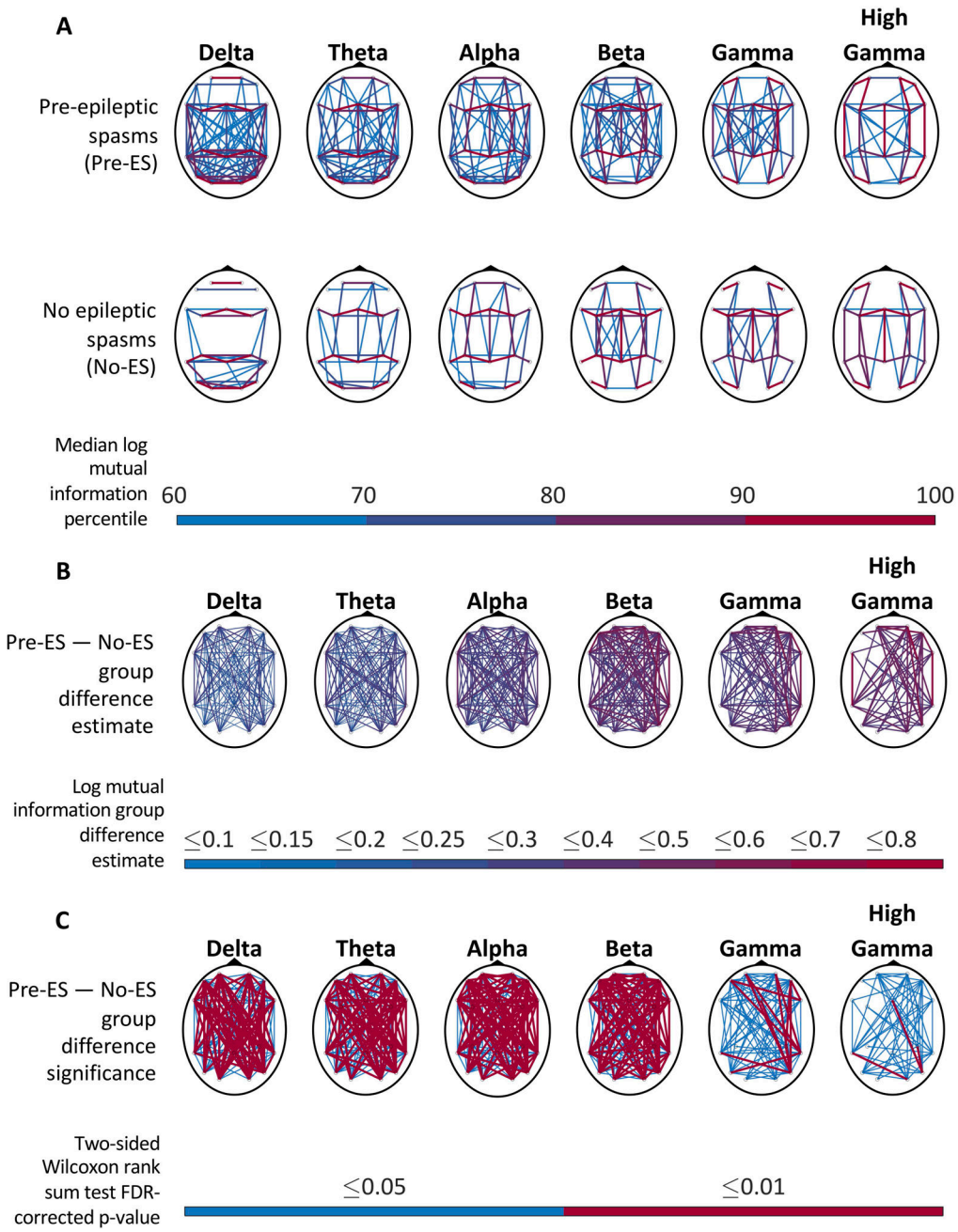

Figure 2.

A. Graphs showing natural log of mutual information (log MI) connectivity in pre-epileptic spasms group (Pre-ES) and no epileptic spasms group (No-ES) in delta, theta, alpha, beta, gamma, and high gamma frequency bands. Anterior channels are at the top of each diagram. Line color and thickness represents median log MI percentile between each pair of channels across all subjects in each group. Median log MI connection values of 60th percentile and higher are shown. Percentiles were calculated within each frequency band across all subjects in both groups. Note that there are more moderate-strength log MI connections (60–90th percentile) in the Pre-ES group.

B. Group comparisons show higher log MI connectivity in the Pre-ES group than the No-ES group in all frequency bands. Line color and thickness represents estimated group difference between Pre-ES and No-ES subjects in log MI between each pair of channels using an ANCOVA model adjusting for age. Only estimates with false discovery rate-corrected (FDR-corrected) p-value ≤.05 are shown. Note that the higher MI connections in the Pre-ES group were mostly between spatially-distant channels, both within and between hemispheres.

C. Statistical significance of estimated group differences in log MI. Line color and thickness represents two-sided Wilcoxon rank sum test FDR-corrected p-value for estimated group differences in log MI between each pair of channels. Note that while lower frequencies had smaller estimated group differences, they had more between-channel differences with higher statistical significance.

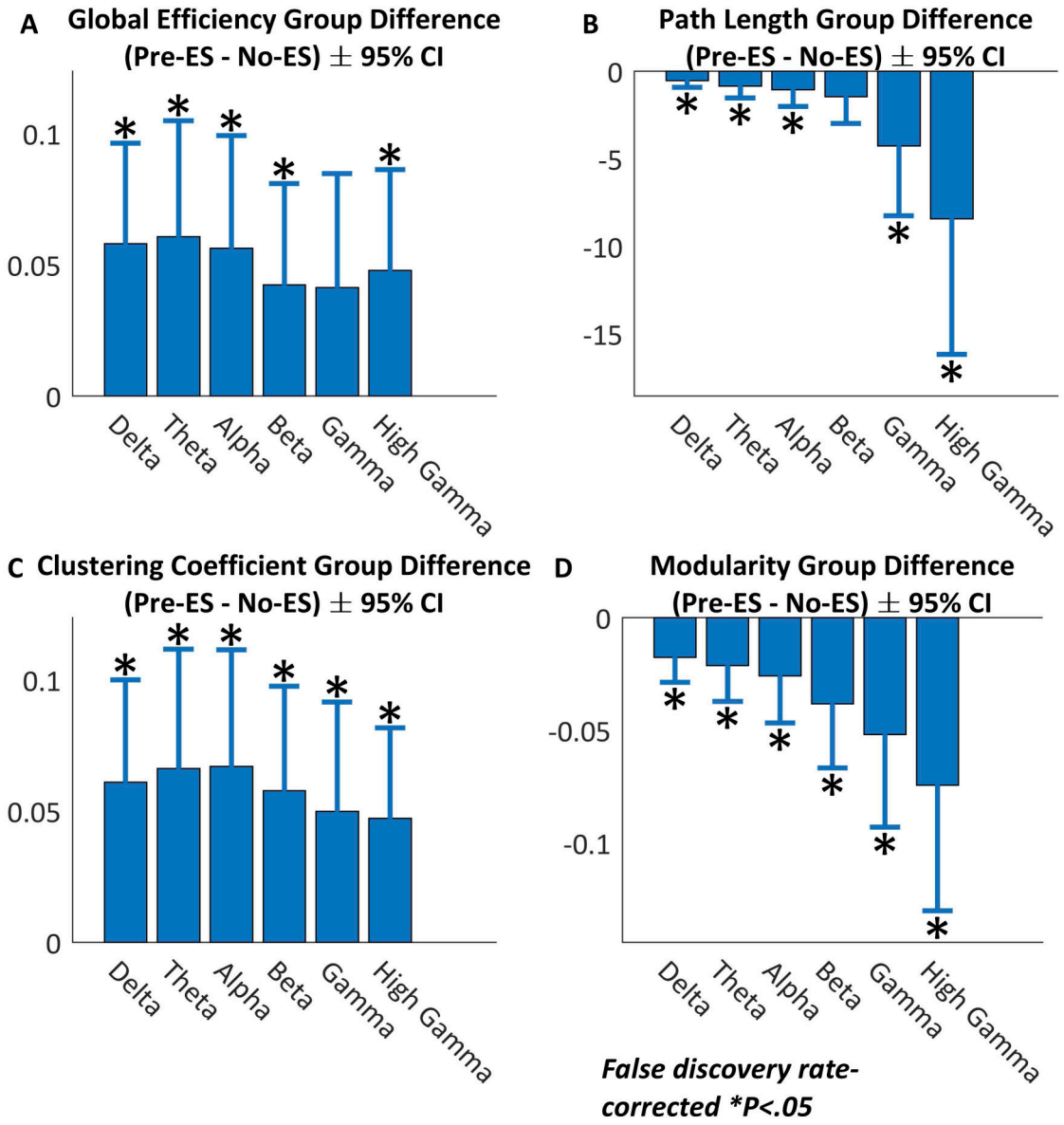

Figure 3.

Graph measures group differences with 95% confidence intervals (95% CI) between pre-epileptic spasms group (Pre-ES) and no epileptic spasms group (No-ES), adjusted for age, in all frequency bands.

A. Global efficiency was significantly higher in the Pre-ES group in delta, theta, alpha, beta, and high gamma frequency bands.

B. Characteristic path length was significantly shorter in the Pre-ES group in delta, theta, alpha, gamma, and high gamma frequency bands.

C. Average clustering coefficient was significantly higher in the Pre-ES group in all frequency bands.

D. Modularity was significantly lower in the Pre-ES group in all frequency bands.

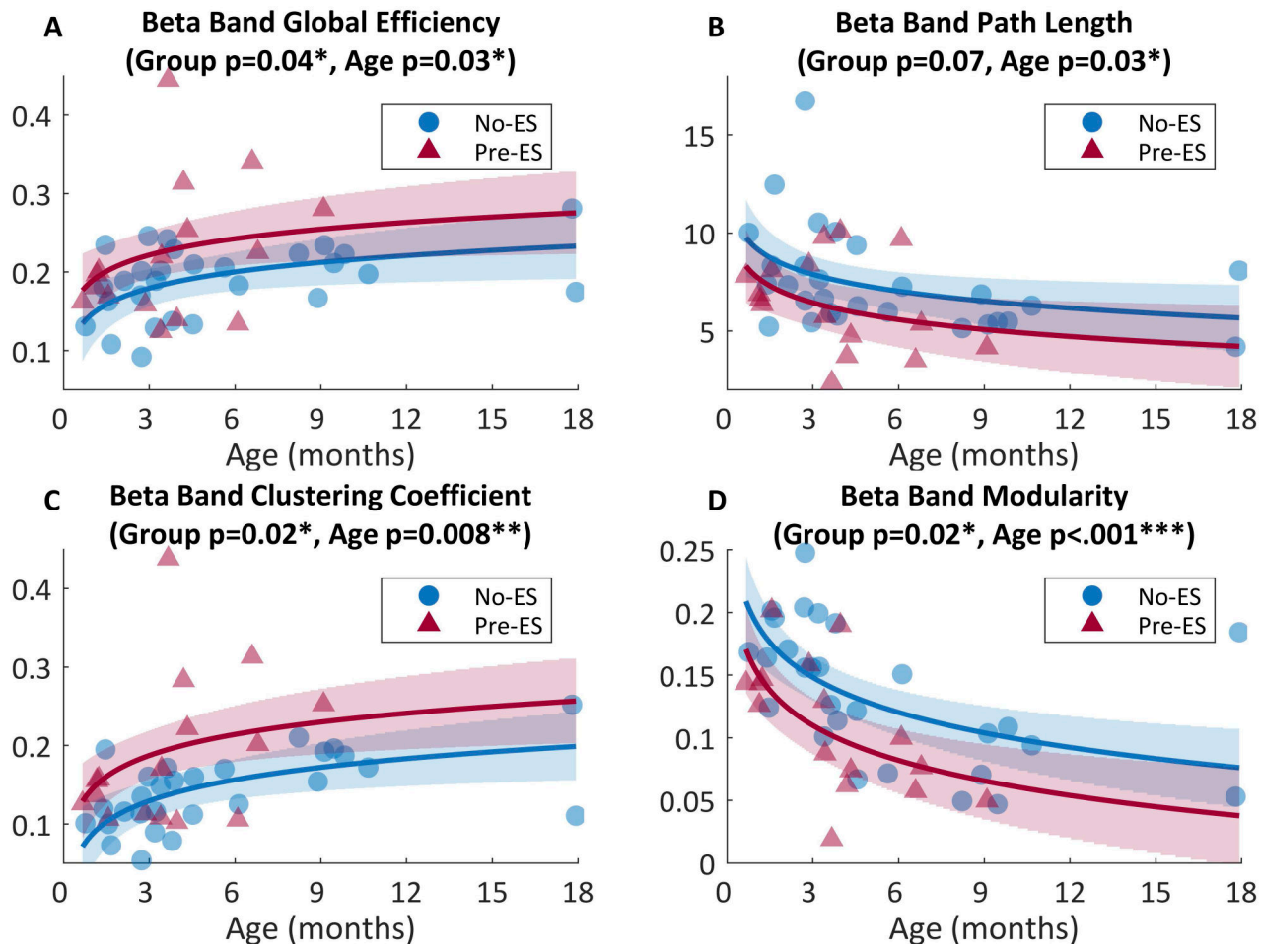

Figure 4.

Scatter plots of graph measures versus log age in the beta frequency band with the estimated fit for each group (Pre-ES in red triangles and No-ES in blue circles) determined using ANCOVA models. The horizontal axis was re-drawn with a linear scale for easier visualization of age in months. The statistical significance of the estimated graph measure group difference and correlation of graph measure with age are shown in the figure titles (false discovery rate-corrected *p<.05, **p<.01, ***p<.001). The shaded areas represent the 95% confidence intervals for each group estimate.

A. Beta frequency band global efficiency vs age. The Pre-ES group has higher global efficiency than the No-ES group. Both groups show a positive correlation of global efficiency with age.

B. Beta frequency band characteristic path length vs age. The Pre-ES group has shorter characteristic path lengths than the No-ES group, but the difference was not statistically significant. Both groups show a negative correlation of characteristic path length with age.

C. Beta frequency band average clustering coefficient vs age. The Pre-ES group has higher average clustering coefficient than the No-ES group. Both groups show a positive correlation of average clustering coefficient with age.

D. Beta frequency band modularity vs age. The Pre-ES group has lower modularity than the No-ES group. Both groups show a negative correlation of modularity with age.

Table 1.

Subject demographics and clinical data

	No-ES (n=28)	**Pre-ES (n=16)**	**p-value**
Number Female (%)	13 (46%)	5 (31%)	.32 (*chi-square* test)
TSC genetic variant (if known)	6 *TSC1* 14 *TSC2* 4 NMI	0 *TSC1* 14 *TSC2* 0 NMI	**.01 (Fisher's exact test *)**
Median age at EEG Days (range)	112 (23 – 538)	106 (20 – 273)	.34 (Wilcoxon rank sum test)
Median age at ES onset Days (range)	–	188 (105 – 449)	–
Median time from first EEG to ES onset Days (range)	–	100 (24 – 197)	–
Number with FS (%)	13 (46%)	10 (62%)	.30 (*chi-square* test)
Median age at FS onset (if present) Days (range)	332 (65 – 603)	448 (1 – 531)	.73 (Wilcoxon rank sum test)
Median time from EEG to FS onset (if present) Days (range, negative indicates EEG after FS onset)	157 (−219 – 419)	248 (−109 – 477)	.29 (Wilcoxon rank sum test)
Number with focal interictal epileptiform EEG activity (%)	9 (32%)	9 (56%)	.12 (*chi-square* test)
Number with slowing on EEG (%)	3 (11%)	3 (19%)	.65 (Fisher's exact test *)

EEG = electroencephalogram, ES = Epileptic spasms, FS = Focal seizures, NMI = No mutation identified, TSC = tuberous sclerosis complex

* Calculated using Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC