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Strong coupling between slow oscillations and wide fast ripples in children with epileptic spasms; Investigation of Modulation Index and Occurrence Rate

Running title

MI and HFOs in epileptic spasms

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Summary

Objective

Epileptic spasms (ES) often become drug-resistant. To reveal the electrophysiological difference between children with ES (ES+) and without ES (ES-), we compared the occurrence rate (OR) of high frequency oscillations (HFOs) and the modulation index (MI) of coupling between slow and fast oscillations.

In ES+, we hypothesized that 1) pathological HFOs are more widely distributed and 2) slow oscillations show stronger coupling with pathological HFOs than in ES-.

Methods

We retrospectively reviewed 24 children with drug-resistant multilobar onset epilepsy, who underwent intracranial video EEG prior to multilobar resections. We measured the OR of HFOs and determined the electrodes with a high rate of HFOs by cluster analysis. We calculated MI, which reflects the degree of coupling between HFO (Ripple/Fast Ripple [FR]) amplitude and five different frequency bands of delta and theta activities (0.5-1Hz; 1-2Hz; 2-3Hz; 3-4Hz; 4-8Hz).

Results

In ES+ (n=10), the $OR_{(FRs)}$, the number of electrodes with high-rate FRs and the $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ in all electrodes, were significantly higher than in ES- (n=14).

In both ES+ and ES- groups, $MI_{(Ripples/FRs\ \&\ 3\text{-}4Hz)}$ was the highest among the five frequency bands. Within the good seizure outcome group, the $OR_{(FRs)}$ and the $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ in the resected area in ES+ were significantly higher than in ES- ($OR_{(FRs)}$, p=0.04; $MI_{(FRs\ \&\ 3\text{-}4Hz)}$, p=0.04).

Significance

In ES+, the larger number of high-rate FR electrodes indicates more widespread epileptogenicity than in ES-. High values of $OR_{(FRs)}$ and $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ in ES+ compared to ES- are a signature of the severity of epileptogenicity. We proved that ES+ children who achieved seizure freedom following multilobar resections exhibited strong coupling between slow oscillations and FRs.

Introduction

1, Epileptic spasms

Epileptic spasms (ES) are characterized by a brief and sudden flexion, extension or mixed movement, either symmetric or asymmetric, lasting for 1-2s[1]. ES are the major subgroup of seizure types in infants from 0 to 2 years[2]. The underlying mechanism for the generation of ES is not yet known. International League Against Epilepsy (ILAE) 2017 classification of seizure types categorized ES into focal, generalized or combined onset seizure type[3]. The cessation of drug-resistant ES after multilobar resection including subtotal hemispherectomy indicates that one of the mechanisms of ES is the focal onset seizure of cortical origin[4-7]. Barba et al. reported 58 patients with ES who underwent unilobar resection and 22 patients with ES who underwent multilobar resection. Forty-nine out of the 80 patients became seizure free[8].

There have been no reports comparing the electrophysiological findings between ES (ES+ group) and focal seizures without ES (ES- group).

2, High frequency oscillations

High frequency oscillations (HFOs) are classified into ripples at 80-200Hz and fast ripples (FRs) at >200/250Hz[9]. HFOs, especially FRs, are estimated to be a potential marker for epileptogenicity[10-13]. A meta-analysis of 11 papers by Höller et al. provides evidence of a higher resection ratio of ripples (10 studies) and/or FRs (7 studies) in seizure-free patients compared with non-seizure free patients[13]. Kobayashi et al. reported ictal fast oscillations (40-150Hz) on the scalp video electroencephalogram (SVEEG) in infantile spasm patients to demonstrate the cortical origin of ES[14,15]. There have been no reports examining HFOs on intracranial EEG comparing ES+ and ES-.

3, Modulation Index

Modulation Index (MI) is one of the parameters that represents the strength of phase-amplitude coupling between the HFO amplitude and the slow oscillations[16]. Pathological HFOs may be preferentially coupled with slow oscillations at 3–4Hz more than with slow oscillations at 0.5–1Hz, which are more often coupled with physiological HFOs[17]. $MI_{(Ripples/FRs\ \&\ 3–4Hz)}$ has been shown to differentiate the multilobar epileptogenic zones from the motor area[7].

4, Hypothesis

We tested the hypotheses that in children with ES+, as compared to children with ES-, 1) pathological HFOs are more widely distributed; 2) slow oscillations show stronger coupling with pathological HFOs. The objective of our study is to elucidate the extent and the severity of the epileptogenicity in the ES+ group vs the ES- group.

Methods

1, Patients

We retrospectively reviewed 24 children with drug-resistant multilobar onset epilepsy who underwent IVEEG recordings for presurgical evaluation between June 2009 and December 2013 at the Hospital for Sick Children. Before surgery, all 24 children underwent SVEEG, magnetic resonance imaging (MRI), and magnetoencephalography (MEG). We assessed 10 ES+ children and 14 ES- children. This study was approved by the Research Ethics Board of the Hospital for Sick Children.

2, IVEEG recording

We selected the region to implant the subdural grid electrodes based on the seizure semiology, ictal/interictal data on SVEEG, MRI lesions, and MEG spike dipoles. We performed IVEEG with subdural grid electrodes and several strip and/or depth electrodes. We implanted the intracranial electrodes in the same way as reported previously[12,18]. We used subdural grid electrodes (diameter: 4mm; exposure: 2.3mm; effective surface area: 4.2mm^2; spacing: 9-13mm) and depth electrodes (effective surface area: 8.3mm^2; spacing: 7mm) (Ad-Tech Medical Instrument, Racine, WI, U.S.A.). We recorded IVEEG by using HARMONY 5.4 (Stellate, Montreal, PQ, Canada) at a sampling rate of 1 kHz (n=6) or 2 kHz (n=18). Our IVEEG data were acquired using 72-121 (mean ± SD; 106 ± 13) electrodes for 46-98 hours (mean; 78 hours).

3, Determination of resection margin

We determined the resection margin based on the visual and spectral analyses of interictal epileptiform discharges on IVEEG, ictal findings on IVEEG, equivalent current dipoles

for the interictal epileptiform discharges on MEG, and the location of eloquent areas[18]. The data in this study were retrospectively analyzed, thus the results of this study did not affect the real-time surgical decision making.

4, EEG selection for analysis

We selected five epochs of 5-minute interictal IVEEG during slow-wave sleep[19]. The five epochs were remote from each other and from seizures by at least one hour. Interictal HFOs can be observed most frequently during non-rapid eye movement (NREM) sleep[20,21].

5, Seizure outcome

Postsurgical seizure outcome was evaluated according to ILAE classification[22]. We defined the children with class I to II as the "good seizure outcome group" and the children with class III to VI as the "poor seizure outcome group".

6, Occurrence Rate (OR) of HFOs

We detected interictal ripples (80–200 Hz) and FRs (>200 Hz) during slow wave sleep using bipolar montage with pairs of two adjacent electrodes in successive numbers connected, excluding the reference electrodes and those with significant artifact[23]. We visually inspected each EEG epoch to ensure that they were not contaminated by significant artifacts, such as environmental or muscle artifacts. We acquired the OR of ripples ($OR_{(Ripples)}$) and the OR of FRs ($OR_{(FRs)}$) using the automated detection of HFOs by MATLAB (R2011b, The MathWorks, Natick, MA, U.S.A.)[12] (Figure S1). We generated topographic maps demonstrating the distribution of $OR_{(Ripples/FRs)}$ (Figure 1). We acquired 5 values of $OR_{(Ripples/FRs)}$ for each electrode by the analysis of 5 interictal

EEG epochs. We calculated the mean of the 5 $OR_{(Ripples/FRs)}$ per each electrode to demonstrate the $OR_{(Ripples/FRs)}$.

We categorized the electrodes into three clusters: high-rate ripples/FRs, medium-rate ripples/FRs and low-rate ripples/FRs, according to the $OR_{(Ripples/FRs)}$ by Ward's method, which is a frequently used hierarchical clustering method, as described in a previous report[24]. We subsequently calculated the number of electrodes with high-rate ripples/FRs (Figure 2).

Between the ES+ and ES- groups, we compared 1) the total number of electrodes; 2) the $OR_{(Ripples/FRs)}$ in all electrodes and 3) the number of electrodes with high-rate ripples/FRs. We also investigated the influence of age at surgery on $OR_{(Ripples/FRs)}$ in all electrodes.

Within the good seizure outcome group, we compared 1) the number of electrodes in the resected/unresected areas and 2) the $OR_{(Ripples/FRs)}$ in the resected/unresected areas between the ES+ and ES- groups.

7, Modulation Index

MI was calculated in an automated fashion at each electrode using EEGLAB Toolbox PACTv.0.17 as described in a previous report[7,17].

We measured the MI between two HFOs (Ripples/FRs) and five different frequency bands of delta and theta activities (0.5-1Hz; 1-2Hz; 2-3Hz; 3-4Hz; 4-8Hz). The MI was analyzed in 1) all electrodes in all 24 children and 2) the resected/unresected area in the 18 children with good seizure outcomes.

Subsequently we compared the $MI_{(Ripples/FRs\ \&\ 3\text{-}4Hz)}$ between the ES+ and ES- groups in 1) all electrodes in all 24 children and 2) the resected/unresected area in the 18 children with

good seizure outcomes.

We also investigated the influence of age at surgery on $MI_{(Ripples/FRs\ \&\ 3\text{-}4Hz)}$ in all electrodes.

We analyzed the correlation between $MI_{(Ripples\ \&\ 3\text{-}4Hz)}$ and $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ in the resected area for each case in the good seizure outcome group to demonstrate the difference of faster HFO coupling with 3-4Hz oscillations between the ES+ and ES- groups.

8, Statistical analysis

All statistical analyses were performed with SPSS Statistics 22 (IBM Corp, Chicago, IL, USA). We performed Mann-Whitney U test between two groups and Steel-Dwass test for intergroup comparisons. Simple linear regression analysis was performed to evaluate the correlation between age at surgery and $OR_{(Ripples/FRs)}$ / $MI_{(Ripples/FRs\ \&\ 3\text{-}4Hz)}$. A level of $p < 0.05$ was accepted as statistically significant.

Results

1, Clinical Profiles

Table 1 summarizes the clinical profiles of 24 children (10 females, 42%). The ES+ group consisted of 10 (42%) children. The ES- group consisted of 14 (58%) children. The age of seizure onset ranged from 0.2 to 6 years (mean 2.7 years) in the ES+ group, and from 0.3 to 14.3 years (5.4 years) in the ES- group. The age of seizure onset in the ES+ group was significantly younger than that in the ES- group (p=0.04).

The age at surgery ranged from 2.8 to 17 years (7.6 years) in the ES+ group, and from 1.8 to 17.7 years (12.2 years) in the ES- group. The age at surgery in the ES+ group was significantly younger than that in the ES- group (p=0.01).

Two children (20%, #2, #9) in the ES+ group and two children (14%, #6, #7) in the ES- group had a history of infantile spasms. There were six (60%) children in the ES+ group and 13 (93%) children in the ES- group with lesions detected on MRI. MEG showed cluster of MEG spike dipoles in nine (90%) children in the ES+ group and 13 (93%) children in the ES- group.

2, Resective surgery and pathology

Five (50%) children in the ES+ group and eight (57%) children in the ES- group underwent resective surgery in the left hemisphere.

Nine (90%) children in the ES+ group and five (36%) children in the ES- group underwent subtotal hemispherectomy consisting of fronto-temporo-parietal resection skipping the motor area.

In the ES+ group, histopathological examinations demonstrated focal cortical dysplasia (FCD) type I in four (40%) children, III in one (10%), astrogliosis in four (40%), and oligodendrogliosis in one (10%). There was no FCD type II in the ES+ group.

In the ES- group, histopathological examinations demonstrated FCD type I in one (7%) child, II in three (21%), III in one (7%), astrogliosis in one (7%), oligodendrogliosis in three (21%), tuberous sclerosis complex (TSC) in three (21%), and glioneuronal tumor and normal pathology in one (7%).

3, Seizure outcome

The follow-up periods ranged from 14 to 56 months (mean 33 months). ILAE seizure outcome class Ia was achieved in 18 (75%) children consisting of seven (70%) in the ES+ group and 11 (79%) in the ES- group.

4, The number of electrodes compared between the good and poor seizure outcome groups

There were no significant differences in the number of electrodes in all electrodes or resected areas between the good and poor seizure outcome groups in total (good, 18; poor, 6), in ES+ group (good, 7, poor, 3) and in ES- group (good, 11; poor, 3) (Table S2)

5, Comparison between the ES+ and ES- groups in all electrodes

5-1 The number of electrodes

The total number of electrodes ranged from 85 to 121 (106.3 ± 12.6) in the ES+ group and from 72 to 120 (105.2 ± 13.1) in the ES- group. (Table 2).

5-2 The $OR_{(Ripples/FRs)}$

The $OR_{(FRs)}$ in all electrodes [0.6 to 31.2 (8.7 ± 10.4)] in the ES+ group were significantly higher than those [0.4 to 10.2 (3.0 ± 2.7)] in the ES- group (p=0.04) (Table 2). The $OR_{(Ripples)}$ in all electrodes ranged from 0.2 to 43.2 (12.0 ± 12.9) in the ES+ group and from 0.9 to 12.7 (5.2 ± 3.8) in the ES- group.

5-3 The number of electrodes with high-rate HFOs

The number of electrodes with high-rate FRs [4 to 39 (21.2 ± 12.8)] in the ES+ group was significantly higher than that [2 to 40 (10.0 ± 10.5)] in the ES- group (p=0.01) (Table 2). The number of electrodes with high-rate ripples ranged from 4 to 46 (21.3 ± 12.6) in the ES+ group and from 2 to 40 (16.2 ± 11.3) in the ES- group.

5-4 Comparison of the MI between two HFOs (Ripples/FRs) and five different frequency bands of delta and theta activities, in all electrodes

In both the ES+ and ES- groups, $MI_{(Ripples/FRs\ \&\ 3\text{-}4Hz)}$ was the highest among the five

frequency bands (Figure 3). In the ES+ group, $MI_{(Ripples/FRs\ \&\ 3\text{-}4Hz)}$ was significantly higher than $MI_{(Ripples/FRs\ \&\ 0.5\text{-}1Hz)}$ (p=0.04). In the ES- group, there was no significant difference of $MI_{(Ripples/FRs)}$ among the five frequency bands.

5-5 The $MI_{(Ripples/FRs\ \&\ 3\text{-}4Hz)}$

The $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ in all electrodes [0.1 to 3.4 (1.1± 1.0)] in the ES+ group was significantly higher than that [0.1 to 1.1 (0.4 ± 0.3)] in the ES- group (p=0.03) (Table 2). The $MI_{(Ripples\ \&\ 3\text{-}4Hz)}$ in all electrodes ranged from 1.0 to 27.0 (7.8 ± 8.6) in the ES+ group and from 0.7 to 7.3 (3.2 ± 1.9) in the ES- group.

5-6 Correlation between age at surgery and $OR_{(Ripples/FRs)}$ / $MI_{(Ripples/FRs\ \&\ 3\text{-}4Hz)}$

In the ES+ group, there was no significant correlation between age at surgery and $OR_{(Ripples/FRs)}$ (r=0.25, p=0.49/ r=0.22, p=0.54). In the ES- group, there was no significant correlation between age at surgery and $OR_{(Ripples/FRs)}$ (r=0.19, p=0.51/ r=0.36, p=0.21) (Figure S2).

In the ES+ group, there was no significant correlation between age at surgery and $MI_{(Ripples/FRs\ \&\ 3\text{-}4Hz)}$ (r=0.29, p=0.42/ r=0.21, p=0.56). In the ES- group, there was no significant correlation between age at surgery and $MI_{(Ripples/FRs\ \&\ 3\text{-}4Hz)}$ (r=0.30, p=0.30/ r=0.03, p=0.91).

6, 18 children with good seizure outcomes; Comparison between the ES+ and ES- groups in the resected/unresected areas

6-1 The number of electrodes in the resected/unresected areas

The number of electrodes in the resected area [44 to 94 (68.4 ± 15.6)] in the ES+ group was significantly higher than that [26 to 61 (46.3 ± 12.1)] in the ES- group (p<0.01)

(Table 3).

The number of electrodes in the unresected area [16 to 61 (35.4 ± 14.0)] in the ES+ group was significantly lower than that [14 to 87 (60.0 ± 18.9)] in the ES- group ($p<0.01$).

6-2 The $OR_{(Ripples/FRs)}$ in the resected/unresected areas

The $OR_{(FRs)}$ in the resected area [0.6 to 37.9 (14.7 ± 13.2)] in the ES+ group were significantly higher than those [0.4 to 8.8 (4.6 ± 4.3)] in the ES- group ($p=0.04$) (Table 3). The $OR_{(Ripples)}$ in the resected area ranged from 4.0 to 54.4 (19.0 ± 17.2) in the ES+ group and from 1.0 to 28.6 (10.0 ± 8.6) in the ES- group.

The $OR_{(FRs)}$ in the unresected area ranged from 0.3 to 17.0 (6.3 ± 6.9) in the ES+ group and from 0.4 to 15.8 (2.8 ± 4.4) in the ES- group. The $OR_{(Ripples)}$ in the unresected area ranged from 0.8 to 32.0 (10.8 ± 11.3) in the ES+ group and from 0.6 to 23.8 (4.5 ± 6.8) in the ES- group.

6-3 Comparison of the MI between two HFOs (Ripples/FRs) and five different frequency bands in the good seizure outcome group

Of all values of $MI_{(Ripples/FRs\ \&\ 5\ frequency\ bands)}$, only $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ in the resected area was significantly higher in the ES+ group than in the ES- group ($p=0.04$) (Figure S3) .

In the resected area, $MI_{(Ripples/FRs\ \&\ 3\text{-}4Hz)}$ was the highest in both the ES+ and ES- groups.

In the unresected area, $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ was the highest in both ES+ and ES- groups, $MI_{(Ripples\ \&\ 3\text{-}4Hz)}$ was the highest in the ES+ group and $MI_{(Ripples\ \&\ 1\text{-}2Hz)}$ was the highest in the ES- group. In both ES+ and ES- groups, there was no significant difference of $MI_{(Ripples/FRs)}$ among the five frequency bands.

6-4 The $MI_{(Ripples/FRs\ \&\ 3\text{-}4Hz)}$ in the resected/unresected areas

The $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ in the resected area [0.1 to 4.5 (1.6 ± 1.5)] in the ES+ group was significantly higher than that [0.2 to 1.9 (0.5 ± 0.3)] in the ES- group (p=0.04) (Table 3). The $MI_{(Ripples\ \&\ 3\text{-}4Hz)}$ in the resected area ranged from 2.7 to 33.3 (11.7 ± 11.5) in the ES+ group and from 1.1 to 15.8 (5.2 ± 4.4) in the ES- group.

The $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ in the unresected area ranged from 0.2 to 2.3 (1.3 ± 0.9) in the ES+ group and from 0.1 to 3.3 (0.5 ± 0.9) in the ES- group. The $MI_{(Ripples\ \&\ 3\text{-}4Hz)}$ in the unresected area ranged from 1.4 to 20.8 (9.4 ± 7.9) in the ES+ group and from 0.4 to 11.7 (3.0 ± 3.2) in the ES- group.

6-5 The correlation of $MI_{(Ripples\ \&\ 3\text{-}4Hz)}$ and $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ in the resected area

In the ES+ group, there was a significant correlation between $MI_{(Ripples\ \&\ 3\text{-}4Hz)}$ and $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ (r=0.96) in each child (p<0.01) (Figure S4). $MI_{(Ripples\ \&\ 3\text{-}4Hz)}$ and $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ (r=0.88) in each child (p<0.01). No overlapping of the 95% confidence interval between the ES+ and ES- groups was found. These findings revealed that FRs coupled more strongly with 3-4Hz oscillations than ripples did in the ES+ group, in comparison to the ES- group.

Discussion

1, Summary of findings

In the ES+ group (n=10), the $OR_{(FRs)}$, the number of electrodes with high-rate FRs and the $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ in all electrodes , were significantly higher than those in the ES- group (n=14).

Within the good seizure outcome group (n=18), the $OR_{(FRs)}$ and the $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ in the

resected area in the ES+ group (n=7) was significantly higher than those in the ES- group (n=11).

2, Widespread epileptogenesis in ES

The number of total electrodes with high-rate FRs in the ES+ group was significantly higher than that in the ES- group.

Chugani et al. reported 50 of 65 patients with ES who underwent hemispherectomy, subtotal hemispherectomy or multilobar resection based on PET studies at Children's Hospital of Michigan between 1993 and 2014[6]. Thirty-seven (74%) of these patients achieved seizure freedom. In another study, 15 of 51 patients with ES secondary to FCD on MRI underwent the multilobar resections[25]. Twelve (80%) of these patients became seizure free. It has been demonstrated that a subset of children with drug-resistant ES, who underwent more widespread resections are more likely to achieve seizure freedom than those who undergo limited resections, such as lesionectomy[5]. Intracranial EEG in 11 children with drug-resistant ES confirmed that focal seizures associated with spasms might originate from multiple cortical areas[26]. The analysis of interictal HFOs using IVEEG suggested that the seizure onset zone was larger in ES than focal seizures[12]. Resection of the areas demonstrating ictal HFOs, especially FRs, on IVEEG correlated with a good surgical outcome in children with ES[27].

In our cohort, electrodes with high-rate FRs in the ES+ group were widely distributed over multiple lobes in the unilateral hemisphere, indicating widespread epileptogenicity.

3, Severity of epileptogenesis in ES

In the ES+ group, $OR_{(FRs)}$ and $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ in the resected area were significantly higher

than in the ES- group. FRs were more prominent in both OR and MI than ripples in the ES+ group.

FRs have been proposed as a biomarker of the epileptogenic zone[9,28]. The interictal high-rate FRs are a possible surrogate marker of the epileptogenic zone[12]. Resection of brain regions containing HFOs, especially FRs, correlated with good seizure outcome using extraoperative electrocorticography in adult patients[29] and intraoperative electrocorticography in pediatric patients[11]. Our data suggested that $OR_{(FRs)}$ in the ES+ group were significantly higher than those in the ES- group. High values of $OR_{(FRs)}$ could be a marker of the severity of epileptogenesis in ES.

MI demonstrates the degree of coupling between the amplitude of HFOs and the phase of slow waves[30]. Nonoda et al. reported that epileptogenic HFOs may be more preferentially coupled with slow waves of 3–4 Hz[17]. We reported that $OR_{(HFOs)}$ and $MI_{(HFOs\ \&\ 3–4Hz)}$ could be valuable biomarkers to identify epileptogenic extra-motor areas[7]. We analyzed the correlations between $MI_{(Ripples\ \&\ 3\text{-}4Hz)}$ and $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ at each electrode. In the ES+ group, FRs coupled more strongly with 3-4Hz oscillations than ripples did, in comparison to the ES- group. The high values of $OR_{(FRs)}$ and $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ represent severe epileptogenicity in drug-resistant ES.

4, Mechanism of ES

ES can be categorized as focal, generalized or of combined onset in the ILAE seizure type classification[3]. Based on our results and previous studies[1,4,6,15,26], we may speculate the two neurophysiological mechanisms of ES in which multilobar resections achieved seizure freedom; 1) ES originates from multifocal cortices, 2) ES originates from

combined cortex and subcortical regions.

1) The cortex

The cerebral cortex has been reported to be the generator of ES[4,6,15,26]. Intracranial EEG showed that ES originate from multiple cortical areas[4,26]. Scalp EEG demonstrated ictal fast oscillations indicative of the cortical origin of ES[14,31]. Our data suggested that extensive resected area showing high values of $OR_{(FRs)}$ and $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ achieved seizure freedom in children with drug-resistant ES.

2) The cortex and the subcortical region

Our study revealed significantly higher value of MI between FRs and slow oscillations in children with ES compared to children without ES. MI reflects the degree of stability of phase–amplitude coupling between HFOs and slow oscillations[30]. Previous studies present slow oscillations as an important feature of ictal and interictal EEG in ES[1,14,24,32]. We speculate that the slow oscillation component may associate with the difference between ES+ and ES-. In patients with ES, who underwent corpus callosotomy, there were no significant changes in the bilateral distribution or any parameters of the slow waves[33]. The slow waves in ES did not originate from the corticocortical pathway via the corpus callosum, but rather from the cortico-subcortico-cortical pathway. Vigevano et al. proposed that cortical-subcortical interaction is essential for the generation of infantile spasms[1]. High values of MI in our data may indicate that slow oscillations originating from the subcortical regions play an important role in the generation of ES. Sakuma et al. reported that children with ES had a higher number of oligodendroglia-like cells (OLCs) in the subcortical region including the gray-white

matter junction and white matter than those without ES[34]. They speculated that increased populations of OLCs in the subcortical region could actually be a subset of responsible features for the ES. We speculate that the interaction between the cortex and the subcortical regions provoke ES.

5, Limitations

A small number of children were enrolled in our study because we selected children who underwent multilobar resection. A subset of ES children underwent unilobar resection to control ES[8]. The epileptogenic characteristics of HFOs between multilobar and unilobar resection for ES should be evaluated. We are currently identifying more children with ES+ and ES- to differentiate the electrophysiological specifics of ES from those of focal seizures.

Since adults were not included in our study, we could not compare OR and MI between children and adults. Instead, we have analyzed the correlation of OR/MI and the age of the children (1.8-17.7 years) when they underwent surgery.

We categorized the electrodes into three clusters: high-rate ripples/FRs, medium-rate ripples/FRs and low-rate ripples/FRs, according to the $OR_{(Ripples/FRs)}$ by Ward's method, as it is a commonly used hierarchical clustering procedure. We simply demonstrated the distribution of electrodes according to the occurrence rate of HFOs. We do not indicate that the high-rate HFO cluster is epileptogenic in this study. The issue of thresholding to determine the epileptogenic HFOs is still under discussion[13]. It would be of interest to compare various thresholding techniques to determine which has the highest validity.

Stereotactic EEG in the future has the potential to delineate subcortical electrophysiological activity in ES. Analysis of cortico-cortical evoked potential (CCEP) has demonstrated epileptic networks[35,36] in patients with focal epilepsy. There are no reports investigating ES using CCEP. CCEP may reveal the widespread epileptogenicity of drug-resistant ES.

A recent study, utilizing preferred phase angles of coupling, delineates the epileptic network activities[37]. Further studies considering preferred phase angles of coupling may explain the electrophysiological features and the detailed epileptic network activity of ES.

Key Point Box

- Children with epileptic spasms (ES+) present a significantly larger number of electrodes with high-rate fast ripples
- $MI_{(Ripples/FRs\ \&\ 5\ frequency\ bands)}$ were higher in ES+ than in ES-
- ES+ and ES- children are distinguished by higher values of $OR_{(FRs)}$ and $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ in the resected area that correspond with the severity of epileptogenicity
- Strong coupling of slow oscillations and FRs in ES+ suggests that multilobar resection may be required in a subset of ES children

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Disclosure

None of the authors have any conflict of interest to disclose.

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Figure legends

Fig. 1. Topographic maps of occurrence rate (OR: /min) of high frequency oscillations and the resection area

The topographic maps show the occurrence rates (OR) of ripples (A), and fast ripples (FRs) (B) in case #7 in the ES+ group. 109 electrodes are implanted in total, consisting of 93 subdural grids over the left fronto-parieto-temporal region, two 4-contact strip electrodes placed over the left frontal pole (strip A) and the left superior frontal gyrus (strip B), and two 4-contact depth electrodes (depth C and D) placed in the MRI lesion. The yellow dot lines indicate the resection margin (frontal lobectomy and temporo-parietal corticectomy). 74 electrodes out of 109 were resected. The red closed circles represent the 46 electrodes with high-rate ripples (A), and the 32 electrodes with high-rate FR (B). The white open circles virtually show the electrodes concealed by the dura on strip A, and the electrodes on depths C and D.

Fig. 2. Histogram of electrodes by occurrence rate (OR: /min) of fast ripples (FRs) in ES+ and ES-

The histograms show the distribution of electrodes ranked according to the occurrence rate (OR) of fast ripples (FRs).

Figure 2(A) demonstrates case #7 in the ES+ group and figure 2(B) demonstrates case #13 in the ES- group. The red and yellow lines divide the electrodes into three groups; high-rate FRs, medium-rate FRs and low-rate FRs (defined by Ward's method). The high-rate FR group consists of 32 electrodes out of 109 in case #7 of the ES+ group (A), and 7 electrodes out of 92 in case #13 of the ES- group (B).

Fig. 3. Modulation Index (MI) of five frequency bands in all electrodes in ES+ and ES-

$MI_{(FRs\ \&\ 3\text{-}4Hz)}$ was significantly higher in the ES+ group than in the ES- group (p=0.03) (B). In both ES+ and ES- groups, $MI_{(Ripples/FRs\ \&\ 3\text{-}4Hz)}$ was highest (A, B).

In the ES+ group, $MI_{(Ripples/FRs\ \&\ 3\text{-}4Hz)}$ was significantly higher than $MI_{(Ripples/FRs\ \&\ 0.5\text{-}1Hz)}$ (p=0.04) (A, B). In the ES- group, there was no significant difference of $MI_{(Ripples/FRs)}$ among the five frequency bands (A, B).

Supporting Information Figure S1. Automated detection of high frequency oscillations

Unfiltered interictal EEG of case #7 (ES+ group) in bipolar montage displayed at 5 s/page (A). Automated detection of ripples (B) and fast ripples (FRs) (C). The detected high frequency oscillations are highlighted in pink (B, C).

Supporting Information Figure S2. Influence of age on occurrence rate (OR: /min) and Modulation Index (MI) in all electrodes

In both ES+ and ES- groups, there were no significant correlations between age at

surgery and $OR_{(Ripples/FRs)}$ or $MI_{(Ripples/FRs\ \&\ 3\text{-}4Hz)}$.

Supporting Information Figure S3. Modulation Index (MI) in the resected/unresected areas in ES+ and ES-

$MI_{(FRs\ \&\ 3\text{-}4Hz)}$ in the resected area was significantly higher in the ES+ group than in the ES- group ($p=0.04$). In both ES+ and ES- groups, there was no significant difference of $MI_{(Ripples/FRs)}$ among the five frequency bands.

Supporting Information Figure S4. Correlation of $MI_{(Ripples\ \&\ 3\text{-}4Hz)}$ and $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ in the resected area in ES+ and ES-

In the ES+ group, there was a significant correlation between $MI_{(Ripples\ \&\ 3\text{-}4Hz)}$ and $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ ($r=0.96$) in each child ($p<0.01$). $MI_{(Ripples\ \&\ 3\text{-}4Hz)}$ and $MI_{(FRs\ \&\ 3\text{-}4Hz)}$ ($r=0.88$) in each child ($p<0.01$). No overlapping of the 95% confidence interval between the ES+ and ES- groups was found.

Table 1. Clinical profiles

	Case	Gender	Age at onset* (years)	Age at surgery* (years)	Seizure type	Hemiparesis	MRI lesion	MEG cluster	PET hypo metabolism	Resection		Pathology	Post surgical hemiparesis	Follow-up (months)	Seizure outcome (ILAE)
										Hemisphere	Lobes				
ES+	1	Male	0.3	2.6	ES, Focal	No	Yes	Yes	Yes	L	FTP	Subpial gliosis	Yes	19	5
	2	Female	0.2	3.3	ES, Focal	No	Yes	Yes	NA	R	FTP	Gliosis	No	48	6
	3	Male	3.9	4.9	ES, Focal	No	Yes	No	Yes	L	FTP	FCD IIIA	No	15	1
	4	Male	0.8	5.8	ES, Focal	No	No	Yes	No	L	FTP	FCD I A/ Gliosis	Yes	33	1
	5	Female	3.8	6.3	ES	No	Yes	Yes	NA	R	TPO	FCD I A/ C	No	56	1
	6	Male	4.2	6.6	ES, Focal	No	No	Yes	Yes	R	FTP	Gliosis	No	30	5
	7	Male	5.1	7.4	ES, Focal, 2G, GC	No	No	Yes	Yes	L	FTP	Subpial gliosis	No	15	1
	8	Female	0.9	10.3	ES, Focal	Yes	No	Yes	Yes	R	FTP	FCD I A	Yes	38	1
	9	Female	1.5	11.8	ES, Focal	No	Yes	Yes	Yes	R	FTPO	Oligodendrogliosis	No	32	1
	10	Male	6	17	ES, Focal, 2G, GC	No	Yes	Yes	NA	L	FTO	FCD I A	No	36	1
ES-	1	Female	0.8	1.8	Focal	No	No	Yes	No	R	TP	FCD IIA	No	38	1
	2	Female	0.3	5.3	Focal, 2G	Yes	Yes	Yes	NA	R	TP	Normal	No	44	1
	3	Male	1.5	6.9	Focal, 2G, GC	No	Yes	Yes	NA	L	TPO	TSC	No	52	4
	4	Female	3	11.3	Focal	No	Yes	Yes	NA	L	FTP	Oligodendrogliosis	No	43	1
	5	Male	10	12	Focal	No	Yes	Yes	No	R	TPO	FCD IIA	No	14	4
	6	Male	0.2	12.3	Focal, 2G	No	Yes	Yes	NA	L	FTP	FCD IIB	No	40	1
	7	Female	0.4	12.6	Focal	No	Yes	Yes	Yes	L	FTP	TSC	No	34	4
	8	Male	9	13.8	Focal, 2G	No	Yes	No	Yes	L	TPO	Oligodendrogliosis	No	14	1
	9	Male	8.7	14.7	Focal	No	Yes	Yes	NA	L	FTP	FCD I B	No	40	1
	10	Female	11	15.1	Focal, 2G, GC	No	Yes	Yes	Yes	R	FTP	Oligodendrogliosis	No	30	1
	11	Male	7.3	15.3	Focal, 2G	No	Yes	Yes	NA	R	FT	FCDIIIB/ DNET	No	47	1
	12	Male	6	15.5	Focal, GC	No	Yes	Yes	NA	R	TPO	TSC	No	32	1
	13	Male	14.3	16.5	Focal	No	Yes	Yes	Yes	L	FT	Subpial gliosis	No	16	1
	14	Female	3	17.7	Focal	No	Yes	Yes	NA	L	TO	Glial neuronal tumor	No	23	1

ES, Epileptic spasms; 2G, Secondary generalization; GC, Generalized convulsion seizure; R, Right; L, Left; F, Frontal; T, Temporal; P, Parietal; O, Occipital; FCD, Focal cortical dysplasia; TSC, Tuberous sclerosis complex; DNET, Dysembryoplastic neuroepithelial tumor; ILAE, International league against epilepsy; NA, Not available ; *p<0.05

Table 2. Comparison between ES+ and ES- in all 24 children

	ES+ (n=10)	ES- (n=14)	p-value
Total number of electrodes	106.3 ± 12.6	105.2 ± 13.1	0.42
Occurrence Rate (OR: /min) in all electrodes			
Ripples	12.0 ± 12.9	5.2 ± 3.8	0.06
FRs	8.7 ± 10.4	3.0 ± 2.7	0.04*
Number of electrodes with high-rate HFOs			
Ripples	21.3 ± 12.6	16.2 ± 11.3	0.17
FRs	21.2 ± 12.8	10.0 ± 10.5	0.01*
Modulation Index (MI) in all electrodes			
Ripples & 3-4Hz	7.8 ± 8.6	3.2 ± 1.9	0.06
FRs & 3-4Hz	1.1 ± 1.0	0.4 ± 0.3	0.03*

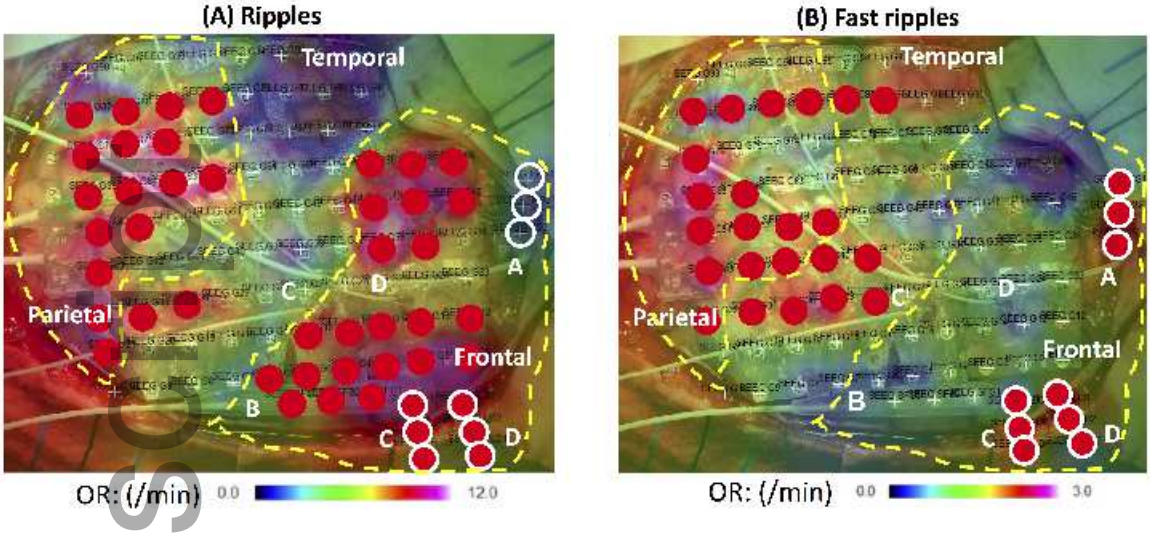
ES, epileptic spasms; FRs, fast ripples; HFOs, high frequency oscillations; *p<0.05

Table 3. Comparison between ES+ and ES- in the good seizure outcome group

	ES+ (n=7)	ES- (n=11)	p-value
Number of lobes including the resected area	3.1 ± 0.4	2.5 ± 0.5	<0.01*
Number of electrodes in the resected area	68.4 ± 15.6	46.3 ± 12.1	<0.01*
Number of electrodes in the unresected area	35.4 ± 14.0	60.0 ± 18.9	<0.01*
Occurrence Rate (OR: /min) in the resected area			
Ripples	19.0 ± 17.2	10.0 ± 8.6	0.11
FRs	14.7 ± 13.2	4.6 ± 4.3	0.04*
Occurrence Rate (OR: /min) in the unresected area			
Ripples	10.8 ± 11.3	4.5 ± 6.8	0.11
FRs	6.3 ± 6.9	2.8 ± 4.4	0.13
Modulation Index (MI) in the resected area			
Ripples & 3-4Hz	11.7 ± 11.5	5.2 ± 4.4	0.09
FRs & 3-4Hz	1.6 ± 1.5	0.5 ± 0.3	0.04*
Modulation Index (MI) in the unresected area			
Ripples & 3-4Hz	9.4 ± 7.9	3.0 ± 3.2	0.06
FRs & 3-4Hz	1.3 ± 0.9	0.5 ± 0.9	0.05

ES, epileptic spasms; FRs, fast ripples; *p<0.05

Topographic maps of occurrence rate (OR: /min) of high frequency oscillations and the resection area



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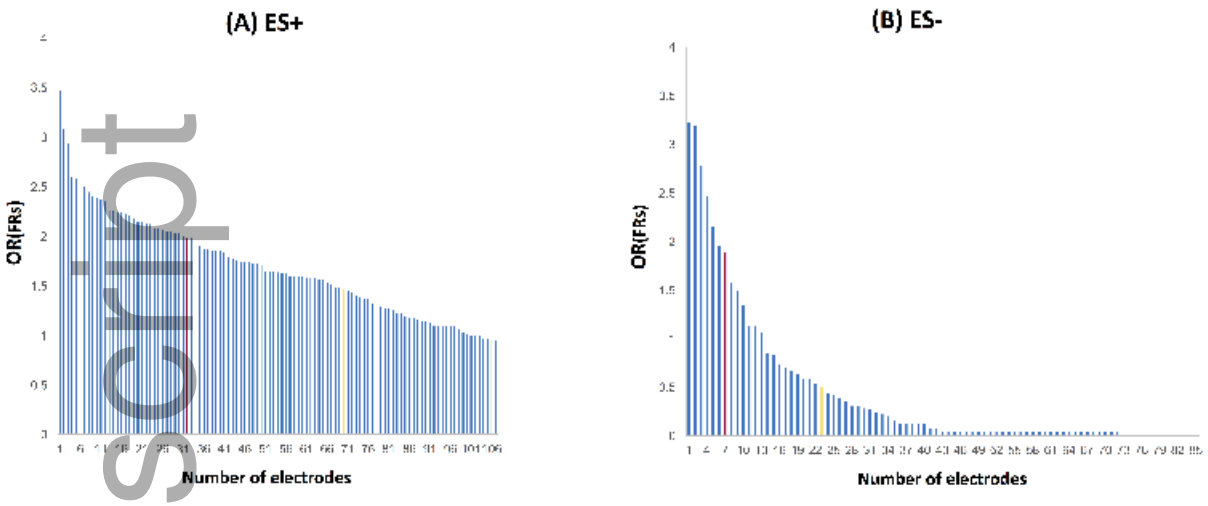

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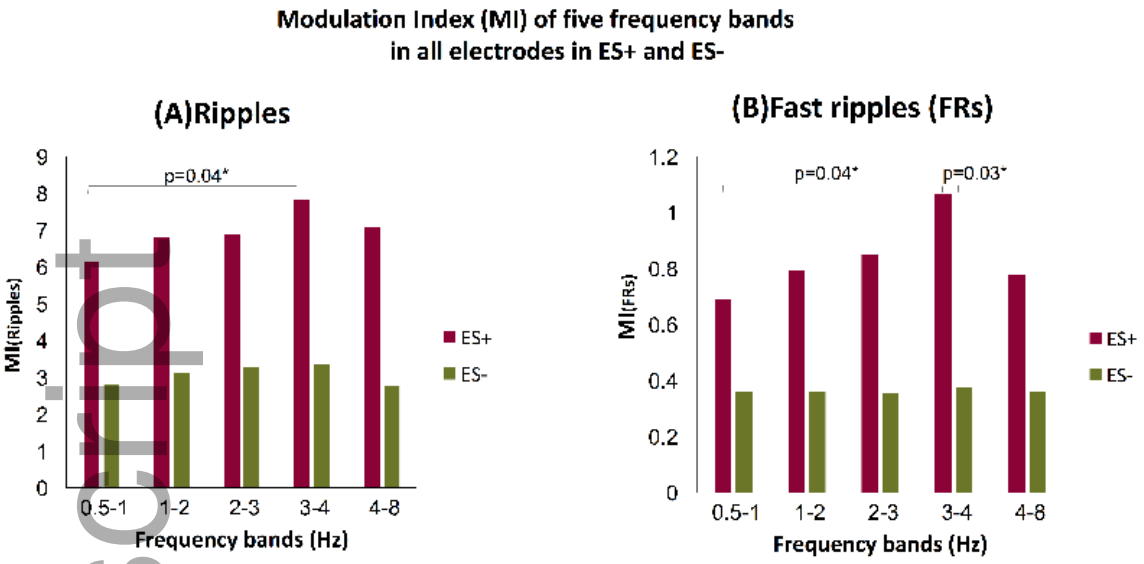

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