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Prospective observational study: Fast ripple localization delineates the epileptogenic zone

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HIGHLIGHTS

- Prospective visual analysis of interictal fast ripples (FRs) was feasible in extra-operative ECoG.
- Interictal FRs was observed beyond the spatial extent of neuroimaging abnormalities in 72% of patients.
- Complete removal of interictal FRs was associated with favorable post-surgical seizure outcome.

ABSTRACT

Objective: To investigate spatial correlation between interictal HFOs and neuroimaging abnormalities, and to determine if complete removal of prospectively identified interictal HFOs correlates with post-surgical seizure-freedom.
Methods: Interictal fast ripples (FRs: 250–500 Hz) in 19 consecutive children with pharmacoresistant focal epilepsy who underwent extra-operative electrocorticography (ECoG) recording were prospectively analyzed. The interictal FRs were sampled at 2000 Hz and were visually identified during 10 min of slow wave sleep. Interictal FRs, MRI and FDG-PET were delineated on patient-specific reconstructed three-dimensional brain MRI.
Results: Interictal FRs were observed in all patients except one. Thirteen out of 18 patients (72%) exhibited FRs beyond the extent of neuroimaging abnormalities. Fifteen of 19 children underwent resective surgery, and survival analysis with log-rank test demonstrated that complete resection of cortical sites showing interictal FRs correlated with longer post-operative seizure-freedom ($p < 0.01$). Complete resection of seizure onset zones (SOZ) also correlated with longer post-operative seizure-freedom ($p = 0.01$), yet complete resection of neuroimaging abnormalities did not ($p = 0.43$).
Conclusions: Prospective visual analysis of interictal FRs was feasible, and it seemed to accurately localize epileptogenic zones.
Significance: Topological extent of epileptogenic region may exceed what is discernible by multimodal neuroimaging.

1. Introduction

Epilepsy is one of the most common neurologic conditions. Of those, 30% of patients are refractory to medications and exhibit increased risk of mortality and neurodevelopmental comorbidities. (Chen et al., 2018; Kwan and Brodie, 2000) Identifying a biomarker

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for intractability and epileptogenic brain regions would be helpful in the evaluation and treatment of epilepsy.

Animal and human studies have demonstrated the presence of high frequency oscillations (HFOs) of 80 Hz and above in the seizure onset zones. (Bragin et al., 1999a; Bragin et al., 1999b; Fisher et al., 1992; Nariai et al., 2011; Staba et al., 2004; Urrestarazu et al., 2007; Worrell et al., 2004) Several retrospective studies have linked favorable post-surgical seizure outcomes to resection of cortical sites showing interictal HFOs, especially fast ripples (FRs) (250 Hz or above) on intra-operative electrocorticography (ECoG) (Hussain et al., 2017; van't Klooster et al., 2017; Wu et al., 2010) and extra-operative monitoring. (Akiyama et al., 2011; Jacobs et al., 2010) Although interictal FRs appear to be a favorable candidate for a biomarker of epileptogenicity, the clinical utility is still limited by the lack of a standardized approach and the challenge of performing the analysis before determination of the resection margin. Only a few prospective studies are available to date, and the results are conflicting. For instance, our prospective cohort of intra-operative ECoG demonstrated complete resection of FRs correlated with favorable post-surgical seizure-freedom (Hussain et al., 2016) and another prospective study showed that complete removal of automatically detected HFOs was associated with post-operative seizure-freedom (Fedele et al., 2017). However, the most recent prospective multi-center study including both intra-operative and extra-operative ECoG failed to validate that complete resection of HFOs is associated with post-operative seizure-freedom. (Jacobs et al., 2018).

Also, the spatial relationship between interictal HFOs and neuroimaging abnormalities (with MRI and FDG-PET hypometabolism) is not well studied. A recent study reported FDG-PET hypometabolism and ictal HFOs are spatially co-localized in temporal lobe epilepsy, but not in extra-temporal lobe epilepsy. (Lamarche et al., 2016) One study has shown abnormalities in stereo-EEG (sEEG) including SOZ, spikes, and slowing did not spatially correlate well with quantitative FDG-PET measurements (Lucignani et al., 1996), suggesting interictal HFOs may not localize well with FDG-PET hypometabolism.

Having established the feasibility of "live" prospective FRs identification (Hussain et al., 2016) as well as favorable inter-rater reliability of visual FRs identification among experienced investigators, (Nariai et al., 2018) we set out to prospectively evaluate the association between HFOs and postoperative seizure outcome in patients undergoing extra-operative ECoG. We also hypothesized that the spatial distribution of interictal FRs is different from the neuroimaging abnormalities and the interictal FRs accurately localize epileptogenic lesions.

2. Methods

2.1. Cohort

Children (below age 21) with medically refractory epilepsy (typically with monthly or greater seizure frequency, and failure of more than three first-line anti-seizure medications) who underwent epilepsy surgery with anticipated cortical resection with the Pediatric Epilepsy Program at UCLA were consecutively recruited between August 2016 and August 2018. Diagnostic stereo-EEG evaluation (not intended for resective surgery) was excluded.

2.2. Standard protocol approvals, registrations, and patient consents

The institutional review board at UCLA approved the use of human subjects and waived the need for written informed consent, as all testing was deemed clinically relevant for patient care. This study was not a clinical trial, and it was not registered in any public registry.

2.3. Patient evaluation

Our procedures for identifying appropriate surgical candidates have been described previously. (Wu et al., 2010) Briefly, all children with medically refractory epilepsy referred during the study period underwent a standardized presurgical evaluation, which—at a minimum—consisted of inpatient video-EEG monitoring, high resolution (3.0 T) brain magnetic resonance imaging (MRI), and ^{18}fluoro-deoxyglucose positron emission tomography (FDG-PET), with MRI-PET co-registration. (Salamon et al., 2008) Clinical characteristics including sex, age at surgery, anti-seizure medications at surgery, duration of the extra-operative monitoring, and the type of epilepsy surgery were documented. The margins and extent of resections were determined according to our standard clinical protocols among the group (consisted of epileptologists, neurosurgeons, radiologists, and neuropsychologists) based on seizure semiology, neurological examination, neuroimaging findings (MRI, FDG-PET, and magnetoencephalography), neuropsychological evaluation (neurocognitive testing, fMRI, and Wada test), non-invasive EEG monitoring, and invasive extra-operative monitoring, which was primarily driven by seizure onset zones. The seizure onset zones were sometimes left behind to prevent an unacceptable neurological deficit.

2.4. Subdural electrode placement

Macroelectrodes, including platinum grid electrodes (10 mm intercontact distance) and depth electrodes (platinum, 5 mm intercontact distance) were surgically implanted. The total number of electrode contacts in each subject ranged from 40 to 128 (median 96 contacts). The placement of intracranial electrodes was guided by the results of scalp video-EEG recording, MRI, and interictal hypometabolism on FDG-PET. All electrode plates were stitched to adjacent plates or the edge of dura mater, or both, to minimize movement of subdural electrodes after placement. In addition, intraoperative pictures were taken with a digital camera before dural closure, to enhance spatial accuracy of electrode display on three-dimensional (3D) brain surface reconstructed from MRI using Brainlab software (Munich, Germany). Upon re-exposure for resective surgery, we visually confirmed that the electrodes have not migrated compared to the digital photo obtained at the electrode implantation surgery.

2.5. Electrocorticography (ECoG) recording

ECoG recording was obtained using Nihon Kohden (Irvine, California, USA). The study recording was acquired with a digital sampling frequency of 2000 Hz, which defaults to a proprietary Nihon Kohden setting of a low frequency filter of 0.016 Hz and a high frequency filter of 600 Hz at the time of acquisition. All ECoGs were part of the clinical EEG recording.

2.6. Visual analysis of fast ripples (FRs)

Prior to surgical resection, each extra-operative ECoG recording was visually reviewed and an artifact-free consecutive 10-min segment during slow-wave sleep was carefully selected for visual analysis of FRs. We selected the segment for analysis at least 2 hours before or after seizures, ideally before anti-seizure medication tapering, and before cortical stimulation mapping, which typically occurred during the second night of monitoring, after anesthesia from implantation had sufficiently worn off. Then, two of three board-certified pediatric electroencephalographers

experienced in visual analysis of HFOs (HN, SAH, and JYW) jointly reviewed the ECoG samples and established a consensus. Using Persyst EEG reviewing software, FRs were marked using bandpass filter between 250–500 Hz, with finite impulse range (FIR) filter settings with time scale of 300 mm/sec. Customized average reference was used for the visual analysis, with removal of electrodes containing significant artifacts. FRs were defined as oscillatory events with at least four cycles and clearly visible above the background signal in the filtered data (see examples in Fig. 1). Marked FR events were then mapped spatially onto each patient's three-dimensional brain surface reconstructed image, along with conventional findings including spikes, slowing, and seizure onset regions (see examples in Figs. 2 and 3). If there was an inter-rater agreement for two or more FR events over 10 minutes localized to a particular electrode, we marked the site as "positive" for FRs. FR event rate (/min) was also recorded. Other conventional neurophysiological findings including spikes, slowing and seizures were marked according to conventional clinical ECoG reading. For each electrode with FR event, the percentage associated a conventional epileptiform discharge was documented.

2.7. Correlation of neuroimaging findings with HFOs

Using the aforementioned findings, each electrophysiologic abnormality (e.g., FRs, spikes, slowing) and the imaging abnormality were manually drawn onto the surface rendering imaging. The MRI abnormality was defined as imaging characteristics of focal cortical dysplasia seen by FLAIR or T2-weighted imaging sequences such as high signal in subcortical white matter, transmantle sign, or gray matter signal increase. When the conventional MRI finding was subtle, PET-MRI co-registration of the hypometabolic area was substituted. Such neuroimaging abnormalities were superimposed onto the reconstructed image after the electrode placement and an experienced neuroradiologist (NS) meticulously determined whether each electrode was within the zone of neuroimaging abnormalities, while blinded to the interictal and ictal ECoG findings, the post-surgical seizure outcome, and the distribution of identified interictal FRs.

2.8. Postoperative assessment

The resection margin was first established in the operating room, with notation of electrodes to be included and excluded from resection, and then verified with review of intra-operative post-resection photographs as well as post-operative MRI. Determination of complete removal of FRs was made as soon as a post-operative brain MRI was available. Follow-up after surgical resection occurred on a routine clinical basis, which typically occurred at 1, 6, 12, and 24 months post-resection. Seizures (if any) were noted at each visit and medications were adjusted when appropriate. We defined "seizure-freedom after surgical resection" at each follow-up visit as the absence of breakthrough seizures since the last clinic visit. Seizures within one week after the surgical resection were not considered breakthrough seizures.

2.9. Statistical analysis

Statistical results were obtained with JMP Pro (version 14; SAS Institute, U.S.A). In this cohort study, the primary outcome was defined as "seizure-freedom after surgical resection at the last follow-up", and the primary exposure of interest was defined as "complete resection of interictal FRs" *a priori*. Other exposures (secondary exposures of interest) included but not limited to: complete resection of seizure onset zones, complete resection of neuroimaging abnormalities, and extension of FRs beyond the neuroimaging abnormality. Wilcoxon rank sum and Fisher's exact tests were used to compare continuous and categorical variables, respectively, between subjects that were seizure free vs. those that were not seizure free at last follow-up. The time to first post-operative seizure was compared between groups (ex. complete vs. incomplete resection) using the log-rank test. Significant results were considered at $p = 0.05$ or less.

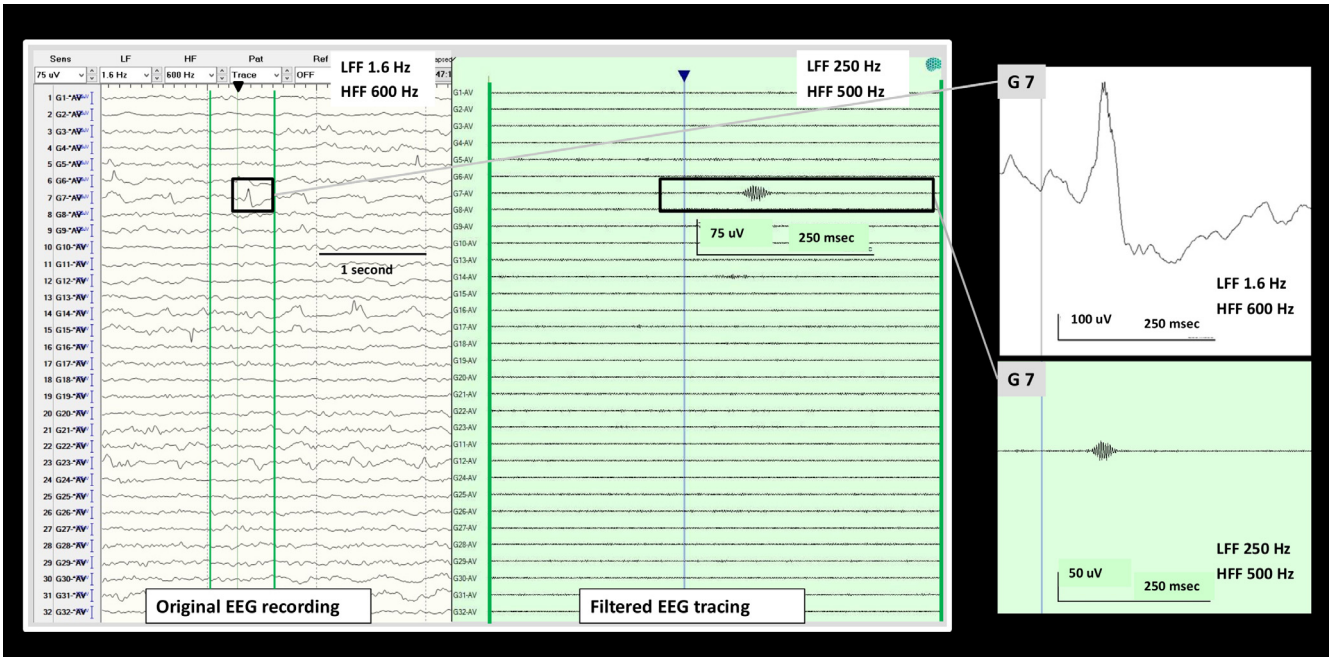

Fig. 1. Example of visual analysis of fast ripples (FRs). An example of visual analysis of fast ripples (FRs) from patient #1. Left: original extra-operative ECoG trace. Right top: temporally expanded original ECoG trace. Right bottom: temporally expanded filtered ECoG tracing for a fast ripple event.

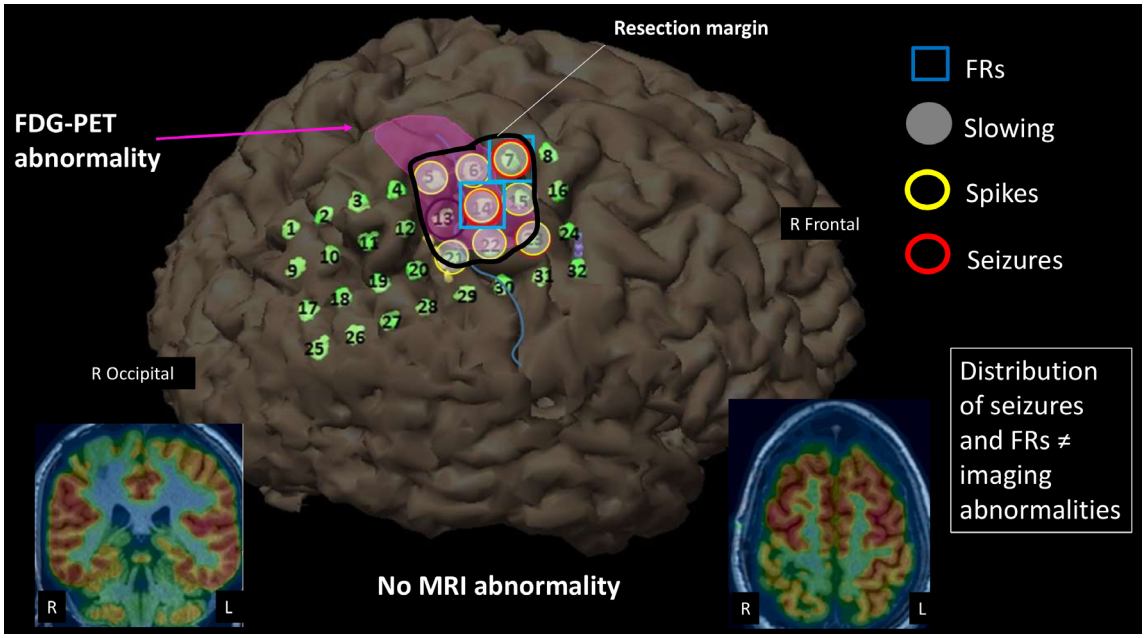

Fig. 2. Example of fast ripples (FRs) and neuroimaging abnormality mapping. An example of FRs and neuroimaging abnormality mapping is shown (patient #1). Distribution of neurophysiological abnormalities including FRs, slowing, spikes, and seizures (onset) is mapped onto the patient's reconstructed three-dimensional brain MRI. A region circled with a pink line indicates FDG-PET hypometabolism noted by a neuroradiologist. There was no structural MRI abnormality noted in this case. Seizure onset zones were removed along with surrounding neuroimaging abnormality. A small portion of FDG-PET hypometabolism was left behind. This patient became seizure-free over 28 months. Please note there was complete overlap of topographic distribution of interictal FRs and seizure onset zones in this case.

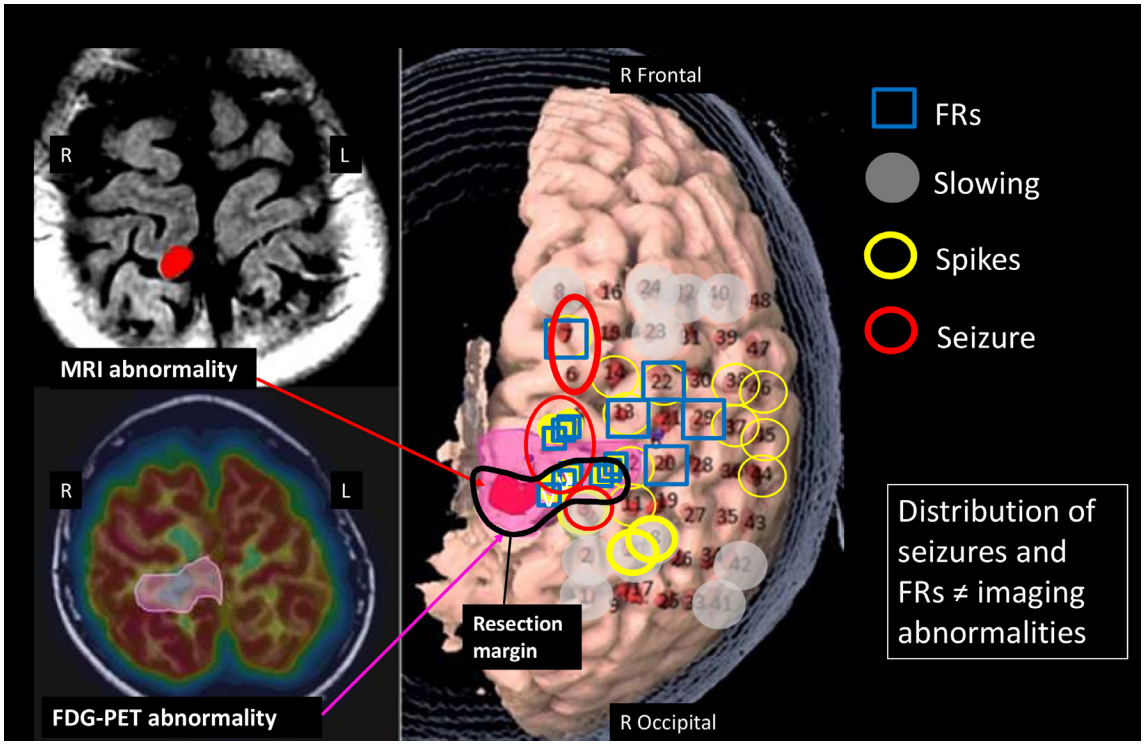

Fig. 3. Example of fast ripples (FRs) and neuroimaging abnormality mapping. An example of FRs and neuroimaging abnormality mapping is shown (patient #9). Distribution of neurophysiological abnormalities including FRs, slowing, spikes, and seizures (onset) is mapped onto the patient's reconstructed three-dimensional brain MRI. A region circled with a pink line indicates FDG-PET hypometabolism, and a small red-shaded area indicates a brain MRI structural abnormality noted by a neuroradiologist. A brain MRI structural abnormality was taken, yet some seizure onset zones and FDG-PET hypometabolism were left behind. This patient had immediate seizure recurrence after the resection. Please note topographic distribution of interictal FRs extends beyond neuroimaging abnormality and localized differently to seizure onset zones.

Table 1
Cohort characteristics.

Pt No.	Sex	Age at surgery (yr)	Epilepsy duration (yr)	Anti-seizure medications	No. of electrodes placed	Duration of EEG (days)	No. of sz captured	MRI lesion/FDG-PET hypometabolism	Surgery	Pathology	No. of electrodes showing FRs	Maximal frequency of FRs (/min)	Complete removal of FR	Complete removal of SOZ	Complete removal of imaging abnormality	FR extends imaging abnormality	All imaging abnormalities covered with electrodes?	Outcome (follow-up in months)
1	M	20	6	CLB, LVT, LCM	40	3	21	NL/R FP	R focal resection of sensorimotor cortex	Gliosis	2	11.0	Y	Y	N	Y	N	Sz free (31 mo)
2	M	11	9	CLB, CNZ, LVT, RFD, PPN	80	3	26	R PO/ R PO	R focal resection around parietal tumor	Ganglioneurocytoma	1	1.3	Y	Y	N	N	N	Sz free (29 mo)
3	F	19	9	LVT, LCM	92	5	8	R F/ R F	R focal resection of frontal cortex	FCD 1b	2	20.0	Y	Y	Y	N	Y	Sz recurrence after 25 mo
4	F	14	10	CLB, LTG, LCM	64	2	7	L F/ L F	L frontal lobectomy sparing sensorimotor cortex	Gliosis	0	0	NA	Y	N	NA	N	Sz free (29 mo)
5	M	9	7	CLB, LTG	100	6	4	R TPO/ R TPO	R TPO	Gliosis	22	0.3	Y	Y	Y	N	N	Sz free (28 mo)
6	F	3	2	CLB, OXC	96	2	18	R F/ R FP	R frontal lobectomy sparing sensorimotor cortex	FCD 2a	2	0.1	N	Y	N	Y	N	Sz recurrence after 1 day
7	M	5	3	PB, PPN, OXC	104	2	22	L FP/ L FP	L frontal lobectomy including resection of sensorimotor cortex	FCD 2a	12	2.3	Y	Y	N	Y	Y	Sz free (27 mo)
8	F	19	7	LVT, LCM	104	2	23	R TPO/ R TPO	No resection (RNS placement of R frontoparietal area)	NA	4	1.7	NA	NA	NA	Y	N	NA
9	M	13	6	OXC, LTG, CLB	72	6	7	R P/ R P	R focal resection of parietal cortex	FCD 2a	7	1.1	N	N	N	Y	Y	Sz recurrence after 10 days
10	F	9	7	CLB, OXC	108	8	35	R F/ R FP	L frontal lobectomy sparing sensorimotor cortex	FCD 1c	24	15.0	N	N	N	Y	Y	Sz free (20 mo)
11	F	8	7	LVT, LCM, CLB	66	6	1	L T/ L TP	L temporal lobectomy	Multinodular and vacuolating neuronal tumor (MVNT)	1	0.1	Y	Y	N	N	N	Sz free (16 mo)
12	F	18	17	CLB, LTG	84	3	25	L P/ L P	L focal resection of parietal cortex	Gliosis	4	0.3	N	N	N	Y	Y	Sz recurrence after 3 days
13	F	15	3	LCM, LVT, OXC	86	4	4	R F/ R F	R focal resection around frontal tumor	Oligodendroglioma	2	0.4	Y	Y	Y	N	Y	Sz free (16 mo)
14	F	19	18	LTG, LCM, OXC, PPN	70	4	37	R FP/ R FP	No resection (RNS placement of R frontoparietal area)	NA	7	0.4	NA	NA	NA	Y	Y	NA
15	F	15	15	TPM, LTG	102	4	4	L TPO/ L TPO	L TPO	FCD 2a, Gliosis	6	0.4	N	Y	Y	Y	N	Sz free (11 mo)
16	M	6	6	CLB, OXC	104	11	2	L PO/ L PO	L parietooccipital resection	Ulegyria, FCD 3d, gliosis	13	28.0	N	N	N	Y	N	Sz recurrence after 23 days
17	M	20	15	LCM, BVC, FBM	118	12	5	L TO/ L TO	L temporal lobectomy plus RNS	Gliosis	7	2.4	N	N	N	Y	N	Sz recurrence after 4 days
18	M	12	5	CNZ, CLP, ECZ, LCM, LVT	112	4	100	L P/ L TP	No resection (RNS placement of L sensorimotor cortex)	NA	6	2.4	NA	NA	NA	Y	Y	NA
19	M	14	6	ECZ, CLB	128	14	8	L T/ L TP	No resection (RNS placement of L temporal, parietal, and occipital area)	NA	5	0.3	NA	NA	NA	Y	N	NA

M: Male; F: Female; FRs: Fast ripples; NA: Not applicable; RNS: Responsive nerve stimulator; FCD: Focal cortical dysplasia; SOZ: Seizure onset zone; Sz: Seizure.
L: Left; R: Right; F: Frontal; P: Parietal; T: Temporal; O: Occipital.
CLB: Clobazam; LVT: Levetiracetam; LCM: Lacosamide; CNZ: Clonazepam; RFD: Rufinamide; PPN: Perampanel; LTG: Lamotrigine; OXC: Oxcarbazepine; PB: Phenobarbital: TPM: Topiramate; BVC: Brivaracetam; FBM: Felbamate; CLP: Clorazepate; ECZ: Eslicarbazepine.

3. Results

3.1. Patient characteristics

There were 19 patients (9 males, 10 females) enrolled during the study period. The median age at surgery was 14 years (range: 3–20 years). Median electrocorticography monitoring duration was 4 days (range: 2–14 days), and median number of seizures captured during the monitoring was 8 (IQ range: 4–25). Details of patient characteristics were listed in Table 1.

3.2. Interictal fast ripples (FRs)

Interictal FRs were observed in 18 out of 19 patients. Among patients with identified FRs, FRs were detected in a median of 5.5 electrodes (range: 1–24), and in all cases, at least one electrode with FRs was co-localized to the seizure onset zones (SOZ). Complete overlap of seizure onset zones and interictal FRs were seen in 3 patients (patient# 1, 2, and 3). Median maximal event rate of interictal FRs across the patients was 1.2/min (interquartile range: 0.3–4.6), and 88% of all FRs were seen with epileptiform discharges. Although median frequency of FRs at seizure onset zones (SOZ) was higher than that of non-SOZ, there was no statistically significant difference between the two groups (median 0.52/min vs. 0.29/min, $p = 0.43$).

Neuroimaging abnormality was covered in its entirety with electrodes in 8 out of 19 patients (42%) (Table 1). At least one of the interictal FRs were seen outside of neuroimaging abnormality in 13 out of 18 cases (72.2%) (see examples in Figs. 2 and 3). In the remaining cases, interictal FRs were seen within neuroimaging abnormality.

3.3. Postoperative outcome

Of 19 patients, 15 underwent resective surgery and the remaining four patients underwent responsive nerve stimulator (RNS) implantation only. One patient (# 17) underwent both resection and RNS implantation. Postoperative seizure outcome was continuously followed. Median post-resection follow-up period was 22 months (range: 9 months–31 months). Eloquent cortices (motor or language areas) overlapping with seizure onset zones or neuroimaging abnormalities were generally spared from resection. Most seizure recurrences occurred between a few days and a few weeks, except one patient (#3, who had complete removal of both FRs and SOZ) who had a seizure recurrence at 25 months after the surgery with different seizure semiology with different scalp EEG onset. None of our patients who had seizures within one week after the surgical resection became seizure-free. Patient #2 and #7 had one episode of seizure-like event 23 months and 24 months after the surgical resection, respectively, but the EEGs did not demonstrate any evidence of seizure. Both were noted as seizure-free at the last clinic visit (29 months and 27 months after the resection, respectively).

Complete vs. incomplete removal of FRs accurately predicted post-operative seizure outcome in 79% (11/14) of the cases, which was same rate as that of complete vs. incomplete removal SOZ (except discordance in patient #6 and #15 – see Table 1).

Univariate comparisons between a group with post-operative seizure-freedom and a group with persistent seizures demonstrated that complete resection of interictal FRs and complete resection of seizure onset zones (SOZ) showed statistically significant differences, yet other variables did not (Table 2). Three out of four (75%) patients who had complete removal of the neuroimaging abnormality achieved post-operative seizure-freedom, and the remaining patient had recurrence of seizures 25 months after the surgery with a different seizure focus on scalp EEG (not contiguous with the prior target). Six out of 11 patients (55%) who had incomplete removal of the neuroimaging abnormality achieved post-operative seizure-freedom.

Survival analysis with log-rank test demonstrated that post-operative seizure-freedom was significantly longer in a group which achieved complete resection of interictal FRs compared to a group which did not ($p < 0.01$). Similarly, post-operative seizure-freedom was significantly longer in a group which achieved complete resection of SOZ compared to a group which did not ($p = 0.01$) (Fig. 4). However, post-operative seizure-freedom duration did not show any difference between a group with complete resection of neuroimaging abnormality (abnormality on either MRI and/or PET hypometabolism) and a group without ($p = 0.43$).

Table 2
Univariate analysis.

	Whole sample	A group with post-operative seizure-freedom* (n = 9)	A group with post-operative seizure recurrence* (n = 6)	p-value
Baseline variables				
Age (yr)	14 (9, 19)	11 (8.5, 15)	15.5 (5.3, 19.3)	p = 0.72
Sex – Male (%)	9 (47%)	4 (44%)	3 (50%)	p = 1.00
Epilepsy duration (years)	7 (6, 10)	7 (4.5, 9.5)	7.5 (5, 15.5)	p = 0.86
Pathology – FCD (%)	7 (47%)	3 (33%)	4 (67%)	p = 0.31
Type of surgery – Multilobar (%)	4 (27%)	3 (33%)	1 (17%)	p = 0.60
Observed number of seizures	8 (4, 25)	7 (4, 24)	7.5 (4, 20)	p = 1.00
Follow-up period (months)	22 (16, 29)	28 (16, 29)	20 (10, 25)	p = 0.22
Primary exposure of interest				
Complete resection of FRs (%)	**7 (50%)**	**6 (75%)**	**1 (17%)**	**p = 0.025**
Secondary exposures of interest				
Complete resection of SOZ (%)	**9 (60%)**	**8 (89%)**	**2 (33%)**	**p = 0.023**
Complete resection of neuroimaging abnormality (%)	4 (27%)	3 (33%)	1 (17%)	p = 0.47
Extension of FR beyond neuroimaging abnormality (%)	9 (64%)	4 (50%)	5 (83%)	p = 0.30
Observed maximal FR frequency (/min)	1.2 (0.3, 4.6)	0.9 (0.3, 8.8)	1.8 (0.3, 22)	p = 0.65

Values are presented with median (IQR) for continuous values, and number (%) for categorical values.
Wilcoxon rank sum test was used to compare continuous values.
Fisher exact test was used to compare categorical values.
* Determination of post-operative seizure-freedom (or recurrence) was determined at the last follow-up.

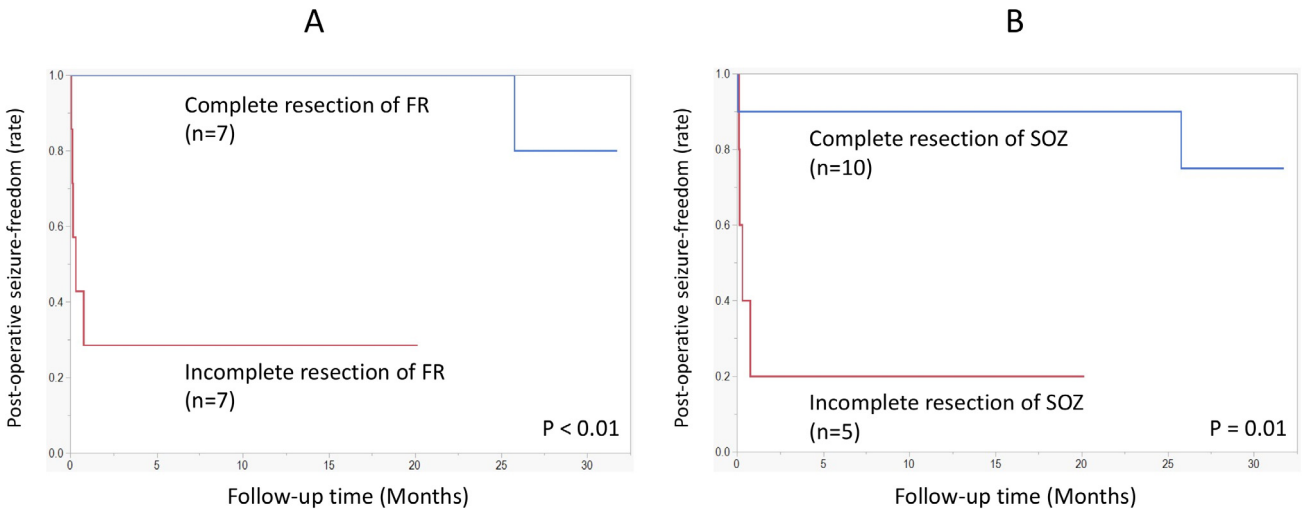

Fig. 4. Survival analysis of the cohort. Survival analysis using Kaplan-Meier curve is shown. (A) Post-operative seizure freedom rate is plotted in the cohort with (blue) or without (red) complete resection of fast ripples (FR). (B) Post-operative seizure freedom rate is plotted in the cohort with (blue) or without (red) complete resection of seizure onset zones (SOZ). P-values were calculated using log-rank test comparing the two survival curves within the cohort. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

We have demonstrated that prospective interictal fast ripple (FR) analysis can be feasibly done in a timely manner as we evaluate patients. In a relatively small cohort, we have again linked complete resection of FRs to favorable post-surgical outcome, extending our prior findings using intra-operative ECoG (Hussain et al., 2016; Hussain et al., 2017; Wu et al., 2010) to a cohort of patients who underwent extended extra-operative ECoG. This is the first prospective observational study to demonstrate complete removal of visually identified FRs is associated with post-operative seizure-freedom in extra-operative ECoG. Notably, only 10-minute of interictal FR analysis from chronic extra-operative ECoG monitoring seemed to accurately predict epileptogenic zones and was comparable with capturing seizure onset zones. This observation is consistent with prior retrospective studies (Akiyama et al., 2011; Jacobs et al., 2010) and one prospective study utilizing automatic HFO detector. (Fedele et al., 2017).

To the best of our knowledge, this is the first study to investigate the spatial relationship between multimodal neuroimaging abnormality (with MRI and FDG-PET hypometabolism) and interictal HFOs. We noted over 70% of interictal FRs were seen beyond the extent of neuroimaging abnormalities. This is consistent with prior reports suggesting neuroimaging abnormalities do not spatially correlate well with intracranial EEG abnormalities. One study demonstrated normal FDG-PET metabolism was found in 62% of the areas with abnormal sEEG activity (SOZ, spikes, or slowing), and FDG-PET hypometabolism was found in 23% of the areas with normal sEEG activity. (Lucignani et al., 1996) Although ictal HFOs were spatially localized with FDG-PET hypometabolism in temporal lobe epilepsy with a group analysis, such correlation was not seen in extra-temporal lobe epilepsy. (Lamarche et al., 2016)

It appears that the spatial distribution of interictal FRs is closely correlated with seizure onset zones, yet not completely overlapping. Neocortical epilepsies often have an extensive epileptic network, and our findings are consistent with prior studies showing wide distribution of interictal and ictal HFOs beyond the extent of the neuroimaging abnormality. (Akiyama et al., 2011; Nariai et al., 2011).

Three out of four (75%) patients who had complete removal of neuroimaging abnormality achieved post-operative seizure-freedom and the remaining patient had recurrence of seizures 25 months after the surgery with a distinctly different seizure focus on scalp EEG. In contrast, 6 out of 11 patients (55%) who had incomplete removal of neuroimaging abnormality achieved post-operative seizure-freedom. Consistent with prior reports, one would aim to achieve complete removal of neuroimaging abnormality if feasible. Having non-invasive neuroimaging information would help determine which brain areas need coverage and recording with intracranial electrodes. However, it is worthwhile to note that post-operative seizure-freedom can be achieved even if some of the neuroimaging abnormality is left behind. Utilizing the spatial information of interictal FRs helps to identify additional areas of the epileptogenic zone not deemed to be abnormal on neuroimaging. Thus, prospective analysis of interictal FRs may be helpful to determine resection margins when it is used in conjunction with multimodal neuroimaging analysis.

Standardized HFO analysis methodology has not been established and inter-rater reliability is a major concern (Frauscher et al., 2017; Spring et al., 2017). The most recent prospective multicenter study including our institution did not demonstrate the association between complete removal of HFOs and seizure outcome as a whole, yet the subgroup analysis of the UCLA cohort did find that complete HFO resection is linked to seizure freedom (Jacobs et al., 2018). We speculate that our consistently favorable results in HFO analysis stem from established and consistent methodology utilized by experienced HFO researchers (Nariai et al., 2018). Also in pediatric epilepsy, neocortical epilepsy due to focal cortical dysplasia (FCD) is a common etiology (Lerner et al., 2009) (in fact 47% of our subjects who underwent resection showed FCD based on pathology). Since HFOs are found more commonly in FCD, (Ferrari-Marinho et al., 2015) utilizing HFOs in pediatric epilepsy surgery may be further justified.

The patient without interictal FRs (patient #4) had a prior history of resective brain surgery, and due to extensive dural adhesions, ECoG sampling was spatially limited and we suspect that characterization of the epileptogenic zone by ECoG was incomplete. However, interictal FRs were found in 18 of 19 patients (95%) in this study, which is superior to the prevalence of interictal FRs in intra-operative studies of 80%, (Hussain et al., 2017; Wu et al., 2010) likely due to the attenuation of anesthesia effects with extra-operative monitoring.

The major limitation of this study is the relatively small cohort, which only included pediatric cases. The choice of pediatric epilepsy is justified in that neocortical epilepsy is more common in children than adults. We performed HFO analysis mainly with

macroelectrodes on grids and strips, as diagnostic stereo-EEG typically does not provide sufficient contiguous coverage of cortical areas that are needed for this study. Also lack of randomization based on the nature of observational study limits ability of establishing the experimental evidence. There was only one patient (#6) in our cohort who had a seizure recurrence after complete removal of seizure onset zones while leaving interictal FRs behind. Even if we knew the result of FR analysis before the resection and we successfully removed the area to achieve post-operative seizure-freedom, our best surgical success would be 67% (10/15 cases of seizure freedom) as opposed to 60% (9/15 seizure freedom). Thus, clinical value of this prospective FR analysis in addition to identifying seizure onset zones during extra-operative monitoring is not clear. Regardless, in order to establish the utility of HFOs in epilepsy surgery, a large prospective multicenter clinical trial is needed.

The neuroimaging abnormalities were superimposed onto the reconstructed image after the electrode placement, then an experienced neuroradiologist meticulously determined whether each electrode was within the zone of neuroimaging abnormalities, while blinded to interictal and ictal ECoG abnormalities, post-surgical seizure outcome, and the distribution of identified interictal FRs. The neuroradiologist thus had knowledge of the electrode locations, which might have introduced bias (ex. towards including neuroimaging abnormality close to the location of electrodes). Precise determination of neuroimaging abnormality before the electrode placement would have been ideal.

Only 42% of cases had complete coverage of neuroimaging abnormality with electrodes, primarily due to craniotomy-limited access (all the cases had craniotomy with strip/grid electrodes placement for resection). We do not have any information as to any FRs outside of electrode coverage. Based on this bias, post-operative seizure-freedom might be better associated with complete resection of brain regions with FRs than that with neuroimaging abnormality.

We performed visual analysis of interictal FRs for 10 min of each recording, consistent with the duration of HFO analysis in prior retrospective and prospective studies from our center. Since it is theoretically possible to observe different spatial distribution of interictal FRs, if the same analysis is performed at different time points in the same patient, (Gliske et al., 2018) we did not consider an electrode to be "positive" with FR until 2 or more events have been identified and localized to the same electrode. Utilizing an automatic HFO detector may have a role, but standardization of methods and verification of detected events remains a significant challenge.

Although 6 of 7 patients who achieved complete resection of interictal FRs became seizure free (one patient had a breakthrough seizure after 25 months, with different seizure semiology and distinct scalp EEG onset), two of 7 patients whose interictal FRs were left behind also became seizure free. A similar observation was reported in the recent prospective study. (Jacobs et al., 2018) This suggests there are interictal FRs which could be left behind, and the presence of physiological HFOs should be considered. Furthermore, disruption of epileptic network by resecting primary epileptogenic region may alter expression of HFOs in other brain regions. A recent study suggests performing HFO analysis in post-resection ECoG better predicts surgical outcome than that in pre-resection ECoG (van't Klooster et al., 2015; van't Klooster et al., 2017). Since we did not perform post-resection ECoG in any of our cases, we cannot verify such findings could be seen in our cases. A better approach may be to utilize HFO findings from both pre-resection and post-resection ECoGs.

We did not evaluate lower frequency HFOs such as ripples (80–250 Hz) in this study. Prior studies showed physiological brain areas including hippocampus, eloquent cortices (visual and sensorimotor cortices) generate HFOs, including ripples and FRs (Frauscher et al., 2018; Melani et al., 2013). Whereas ripples are quite frequent in both physiological and pathological cortices, FRs are much less frequent (the mean rate of FRs was reported as 0.038/min) (Frauscher et al., 2018; Jacobs et al., 2010). Based on prior studies, ripples are less specifically located in epileptogenic regions than FRs. (Akiyama et al., 2011; Jacobs et al., 2010; Staba et al., 2004) Our preliminary analysis also showed abundant ripples from presumed epileptogenic regions as well as eloquent cortices. Although visual quantification of ripples would be challenging due to its high prevalence, utilizing high quality HFO detector, when available, may help determine differences in the topological distribution of ripples and FRs.

Whether our approach to FR identification and localization can be used in different institutions remains to be proven. It is clear that electroencephalographers who are not experienced in visual HFO analysis need to be trained to maximize inter-rater reliability. A prior study failed to show favorable inter-rater reliability on HFO analysis using ECoG samples from extra-operative studies, possibly due to enrollment of electroencephalographers with limited experience in HFO analysis (Spring et al., 2017). In contrast, we recently found high inter-rater reliability of visual analysis of HFOs amongst EEG readers experienced in HFO analysis (Nariai et al., 2018).

Once fully trained in visual analysis of HFOs, the EEG readers can feasibly perform "live" analysis of ECoG prospectively in the operation room (Hussain et al., 2016) as well as during extra-operative monitoring as demonstrated in this study. If training multiple EEG readers for HFO analysis becomes feasible, we will be able to utilize HFO data in both research and clinical arenas, including multi-center clinical trials.

Declaration of Competing Interest

The authors have no conflict of interests to disclose.

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