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High-frequency oscillations in a spectrum of pediatric epilepsies characterized by sleep-activated spikes in scalp EEG

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HIGHLIGHTS

- Scalp ripples were detected from various types of pediatric epilepsies with sleep-activated spikes.
- Sleep spikes were heavily loaded with ripples during young age in the CSWS groups.
- Abundant pathological ripples might affect functions of the immature brain networks.

ABSTRACT

Objective: We studied ripple-band (80–200 Hz) high-frequency oscillations in scalp electroencephalogram (EEG) in various pediatric epilepsies featuring sleep-activated spikes, such as epileptic encephalopathy with continuous spike-and-wave during sleep (CSWS) and investigated their characteristics.
Methods: The subjects were 94 children with epileptic disorders including idiopathic and non-idiopathic CSWS, benign epilepsy with centrotemporal spikes (BECTS), Panayiotopoulos syndrome, other types of focal epilepsies (oFE), and focal spikes without clinical seizures (Latent). We detected ripple oscillations using a semi-automatic detection tool based on localized power increase.
Results: In the idiopathic CSWS Group, the median ratio of ripples per spike in the initial EEG was 5.73, which was significantly higher than those in the BECTS, Panayiotopoulos syndrome, oFE, and Latent Groups (0.39, 0.02, 0.35, 0, respectively, all with $p < 0.01$). Ripples were particularly frequent at younger ages.
Conclusions: This paper is the first to confirm a high ratio of ripples per spike in CSWS in the largest number of patients to date.
Significance: The dense generation of ripples, which occurs through a combination of heavy loading of individual spikes with ripples and large numbers of spikes during sleep, characterizes CSWS and might be closely related to the pathophysiology of this epileptic encephalopathy.

1. Introduction

There is a spectrum of childhood epileptic disorders that are characterized by the age-dependent appearance and disappearance of sleep-activated epileptic discharges with a focal or possibly focal origin in electroencephalogram (EEG). These disorders range from idiopathic, self-limited focal epilepsies, including benign epilepsy with centrotemporal spikes (BECTS) and Panayiotopoulos syndrome, to epileptic encephalopathy with continuous spike-and-wave during sleep (CSWS) (Galanopoulou et al., 2000; Japaridze et al., 2014). Pediatric epilepsies showing the extraordinary EEG abnormalities of CSWS include atypical benign partial epilepsy (ABPE) (Aicardi and Chevrie, 1982) and Landau-Kleffner syndrome (LKS) in addition to epileptic encephalopathy with CSWS, and they may be collectively termed encephalopathy with electrical status epilepticus during slow sleep (ESES)/CSWS syndrome (Tassinari et al., 2000). There are also some children with non-idiopathic focal epilepsies or neuropsychological disorders

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without clinical seizures who exhibit sleep-activated spikes in EEG.

High-frequency oscillations (HFOs) in EEG data are attracting attention due to their close relationship with epileptogenicity, which is possibly even closer than that with epileptic spikes (Jacobs et al., 2010, 2012; Wu et al., 2010; Akiyama et al., 2011; Frauscher et al., 2017). HFOs are therefore regarded as a surrogate biomarker of epileptogenicity, and they include ripples (80–200/250 Hz) and fast ripples (200/250–500/600 Hz). HFOs were initially recorded with microelectrodes (Bragin et al., 1999) and then with clinical intracranial electrodes (Jirsch et al., 2006). They are now detectable even over the scalp (Kobayashi et al., 2010, 2011, 2015; Andrade-Valenca et al., 2011; Worrell et al., 2012). Fast oscillations (FOs) in the gamma (40–80 Hz) and ripple bands over the scalp have been confirmed to correspond to cortical HFOs (von Ellenrieder et al., 2014; Zelmann et al., 2014).

We and others previously reported the observation of ripple oscillations associated with spikes in scalp EEG recorded from children with epileptic encephalopathy with CSWS, ABPE, BECTS, and Panayiotopoulos syndrome (Kobayashi et al., 2010, 2011; Qian et al., 2016; Shibata et al., 2016; Ikemoto et al., 2018). Epileptic FOs are known to mirror disease activity with respect to hypsarrhythmia in West syndrome (Kobayashi et al., 2015) and seizures associated with rolandic spikes (van Klink et al., 2016). To the best of our knowledge, however, no clinical studies have been conducted to directly compare the rate of ripple oscillations among various types of pediatric epilepsies with the common characteristics of sleep-activated spikes. We hypothesized that epileptic encephalopathy with CSWS is associated with much more prolific generation of ripples in terms of total number of occurrences as well as ratio of ripples per spike compared to the other types of related childhood epilepsies.

As per the standard method, HFOs and FOs are normally visually identified in filtered and temporally expanded EEG traces by experienced reviewers (Jacobs et al., 2010, 2012). Such conventional methods of visual detection of HFOs/FOs, however, may be biased due to subjective judgment. In order to introduce objectivity into counting the occurrence of ripples for comparison among disorders, we therefore adopted a semi-automatic detection tool for HFOs/FOs with visual confirmation that was developed and confirmed with respect to its utility (von Ellenrieder et al., 2012). We aimed to clarify the pathophysiology of age-dependent childhood epilepsies by involving a so far largest number of participants from the viewpoint of scalp-recorded HFOs and their longitudinal changes, which should have a serious clinical meaning in these disorders.

2. Subjects and methods

2.1. Subjects

Subjects of the present study were a total of 94 children (55 boys, 39 girls) who exhibited sleep-activated epileptic discharges with a presumed focal origin in scalp EEGs that were initially recorded at age <13 years at Okayama University Hospital between May 22, 2012 and December 31, 2016. Sleep-activated epileptic discharges were rather arbitrarily defined as observation of at least twice as many spikes during sleep than during wakefulness in this study. Clinical information and digitally recorded EEG data before and after the time of consent for involvement in the study were used for the analysis, and the period of data acquisition ranged from January 2004 to December 2016. The present study did not affect the treatment of patients.

According to the definition of idiopathic epilepsy by "no known or suspected etiology other than possible hereditary predisposition" (Commission, 1985; Scheffer et al., 2017), we separated the patients with CSWS into idiopathic (iCSWS) and non-idiopathic (nCSWS) Groups. Ten and four patients were in the iCSWS and nCSWS Groups, respectively. The distinctive features of epileptic encephalopathy with CSWS included a characteristic age of onset with a peak of around 4–5 years, heterogeneous seizure types (such as mostly nocturnal focal motor or generalized seizures, absences, and epileptic negative myoclonus), the typical EEG pattern of CSWS, and a variable neuropsychological regression consisting of intellectual decline, reduction of language, disturbance of behaviors, and motor impairment. We used a spike-wave index (SWI) in the non-rapid eye movement (NREM) sleep EEG ranging from 85 to 100% as the diagnostic criterion for CSWS (Tassinari et al., 2000), and the SWI was investigated in the first EEG record obtained for every patient at Okayama University Hospital.

We diagnosed 19 patients with BECTS (BECTS Group), 16 with Panayiotopoulos syndrome (Panayiotopoulos Group), 23 with other types of focal epilepsies (oFE Group), and the remaining 22 with focal spikes in EEG without clinical seizures (Latent Group). The diagnostic criteria of BECTS includes mostly nocturnal focal motor and/or generalized seizures with the onset ranging from 3 to 13 years of age and centrotemporal (rolandic) spikes with activation during sleep in EEG (Commission 1989). The diagnostic criteria of Panayiotopoulos syndrome include seizures with predominantly autonomic, particularly emetic, symptoms that were often prolonged, prone to occur during sleep and start at 1–14 years of age, and EEG spikes with variable or multiple foci, often with occipital predominance (Ferrie et al., 2006). BECTS and Panayiotopoulos syndrome were idiopathic by definition. The oFE group includes heterogeneous focal epilepsies that lack the CSWS pattern and do not belong to benign epilepsies because of the presence of neurological abnormalities, intellectual deficits, and/or lesions in the brain parenchyma in neuroimaging. The patients with spikes without seizures underwent EEG examination due to various neurological conditions including developmental disorders, tics, headaches, and suspected seizures. The oFE and Latent Groups were presumably non-idiopathic.

This study was approved by the Okayama University Ethics Committee (approval No. 546), and written informed consent was obtained from the families.

2.2. Methods

EEG was recorded with a sampling rate of 500 Hz using the Nihon-Kohden (Tokyo, Japan) Neurofax system. The international 10–20 electrode system was used, and the analysis was performed in Fp1, Fp2, F3, F4, C3, C4, P3, P4, O1, O2, F7, F8, T3, T4, T5, T6, Fz, Cz, and Pz in a referential montage, using the average EEG of the earlobes (A1 and A2) as a reference (indicated as Aav).

In each individual NREM sleep EEG record, we manually selected a 60-s-long data section with no or minimal artifacts. This data length was adopted because it was difficult to obtain a clean consecutive section longer than 1 minute from every EEG record during a nap. First, we automatically detected candidate ripples from a selected data section using a program written by von Ellenrieder et al. (2012) for MATLAB (version 9.1.0; MathWorks Inc., Natick, MA, USA). This program is, in brief, designed to detect oscillations as localized increments of the signal power with a duration of at least four cycles in narrow frequency bands based on a finite impulse response (FIR) filter. In detail according to von Ellenrieder et al., in each frequency band, they compute the root-mean square (RMS) value of the signal in a moving window of duration equal to four cycles of the center frequency of the band (central frequency), and compare it to a moving threshold. An event or candidate oscillation is detected every time the RMS value of the signal is above the threshold during a time interval equal to

four cycles of the central frequency of the band plus the effective duration of the impulse response of the narrowband filters. These filters have an impulse response with a shape that could be confused with an oscillation, and any glitch or fast transient in the broadband signal generates a confounding event in the narrow bands. The duration of these events is equal to the effective duration of the filter response. On the other hand, if an oscillation is present in the broadband signal, when filtered by the narrowband filter, its duration also increases by an amount equal to the effective duration of the impulse response. Then, by selecting only oscillations lasting longer than fours cycles plus this effective duration, they avoid false positives related to fast transients in the original signal.

In the present study, the whole frequency band of analysis ranged from 80 to 200 Hz. It was separated into 10 Hz-wide narrow frequency bands (i.e. 80–90 Hz, 90–100 Hz, 100–110 Hz…190–200 Hz). As spectral analysis was not incorporated in this program, the exact frequency of each ripple could not be measured. Therefore, in each narrow frequency band, the central frequency represented approximate frequency of the detected ripples from this band (e.g. 105 Hz in the 100–110 Hz band). The threshold of RMS power to detect ripples was set as a ratio of 3 against the RMS power level of a background moving window (width 30 s); this ratio was slightly higher than the original value of 2.5, and therefore the detection of ripples was rather conservative in the present study because we hoped to detect definite ripples from pediatric EEG data that tended to have a rather noisy background.

Second, the automatically detected candidate ripples were marked on temporally expanded (2 s per page) and filtered EEG traces using an inhouse written program (overlaid traces with low-cut frequency [LCF] filters at 0.5 and 80 Hz in blue and red, respectively) (e.g., see Fig. 1). Ripples of at least four consecutive oscillations were visually confirmed through the consensus of two reviewers, and oscillations that appeared to be contaminated noise or muscle activity were excluded. Signals common to almost all channels were assumed to be contaminated noise or muscle activity from the reference (Aav).

Third, we evaluated the number of occurrences, frequency (central frequency of the corresponding narrow frequency band of ripple detection), and duration (duration of power increase in the corresponding narrow frequency band) of ripples with or without spikes in each EEG dataset for further analysis. We analyzed ripples separately with respect to association and un-association with visually identified epileptic discharges because HFOs with and without associated spikes may have different meanings regarding epileptogenicity. Ripples on different scalp electrodes were identified separately. We also counted spikes during the selected EEG section to show ratio of ripples per spike. We defined a spike as an event in time, independently of the number of electrodes on which it was observed. Analysis was performed on a yearly basis, and we investigated the changes of parameters regarding ripples across age in each patient group.

2.3. Statistical analysis

Regarding the initial EEG record of individual patients, we statistically compared the number of ripples associated with and without spikes, the number of spikes, the ratio of ripples per spike, and the frequency and duration of ripples between the patient groups using the Steel–Dwass test, a non-parametric multiple comparison test. The initial EEG record referred to the first sleep EEG obtained at Okayama University Hospital. The numbers of ripples with and without spikes in the initial EEG in each patient were compared using the Wilcoxon signed-rank test. We also statistically analyzed age-dependent changes in the parameters of the ripples and spikes between childhood (prior to 10 years of age) and preadolescence and adolescence (after 10 years) using the Wilcoxon signed-rank test in patients who had EEG recordings from both age ranges. Relationships were considered statistically significant if $p < 0.05$. We used the JMP Japanese version 11 (SAS

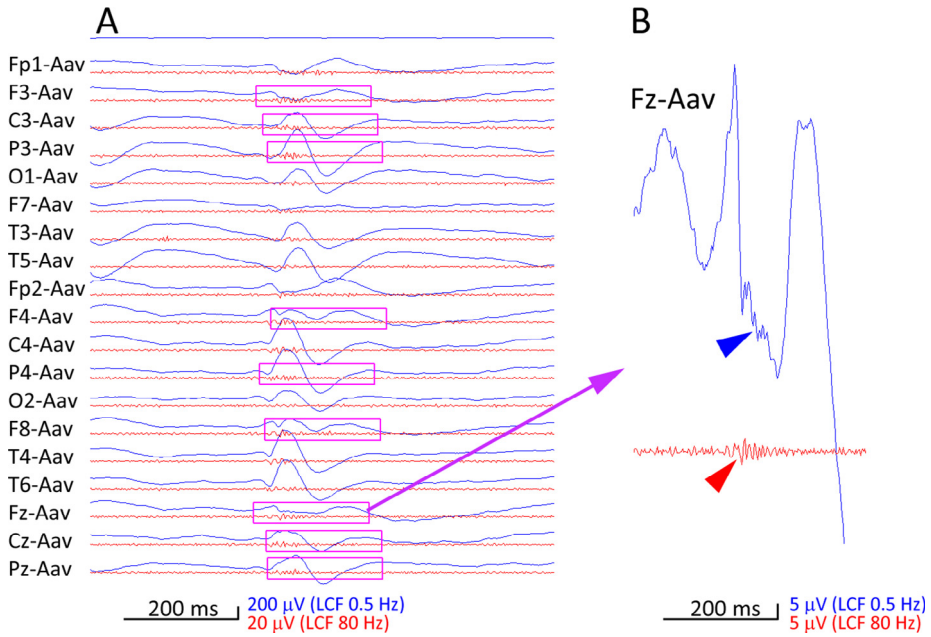

Fig. 1. Ripple oscillations in the scalp electroencephalogram (EEG) recorded from a patient with atypical benign partial epilepsy in the iCSWS Group (case 3 in Table 2). (A) Temporally expanded and overlaid EEG traces are shown with two different low-cut frequency (LCF) filters (0.5 Hz and 80 Hz in blue and red, respectively) using the average EEG of A1 and A2 as a reference (Aav). Ripples of at least four consecutive oscillations associated with spikes are indicated by pink squares. (B) A representative ripple associated with a spike on the Fz channel is shown in magnification; this ripple is indicated by a blue arrowhead in the EEG trace filtered at 0.5 Hz and by a red arrowhead in the trace filtered at 80 Hz.

Institute Japan, Tokyo) for statistical analysis. In the investigation of frequency and duration of ripples, the data from patients who lacked ripples were excluded from this analysis.

3. Results

3.1. Clinical diagnosis

Demographic data of the patients are indicated in Table 1.

We analyzed a total of 481 EEG records in all diagnostic groups. The median ratio of number of spikes during sleep to number of spikes during wakefulness was 5.8 [range: 2.1–55] in 81 patients who showed spikes during wakefulness. In the remaining 13 patients, spikes occurred during sleep but not during wakefulness. The 10 and 4 patients in the iCSWS and nCSWS Groups, respectively, exhibited the CSWS EEG pattern with SWI > 85% (mean 92% and 99%, respectively) at each patient's first examination at Okayama University Hospital (Table 2). All CSWS patients were treated medically. In the iCSWS Group, one and three patients showed clinical features of Panayiotopoulos syndrome (patient 6) and BECTS (patients 2, 8, and 9), respectively, in the initial stage of the disorder, and subsequently exhibited EEG worsening into CSWS; intelligence quotient (IQ) declined during the follow-up period in three (patients 6, 8, and 9) of these four patients, and the remaining patient (patient 2) became irritable in association with the CSWS EEG pattern but rapidly responded to medical treatment without an obvious decline in intelligence. Patient 3, who had absence, atonic, and mainly nocturnal focal motor seizures during the period of CSWS without apparent neuropsychological impairment or abnormal neuroimaging findings, was diagnosed with ABPE. Patient 10 acquired aphasia in association with EEG worsening into the CSWS pattern after the initial stage showing clinical features compatible with BECTS and was diagnosed with LKS. Of the remaining four patients (patients 1, 4, 5, and 7), patient 1 showed hyperkinetic behaviors without evident decline in IQ, and the other three had various degrees of decline in IQ/developmental quotient (DQ) during follow-up. In the nCSWS Group with structural etiologies, all patients showed a remarkable degree of intellectual decline during follow-up.

The 19 and 16 patients in the BECTS and Panayiotopoulos Groups, respectively, showed the typical clinical findings corresponding to their diagnoses. These groups' mean SWIs in the initial EEG record were 29% [range: 5–53%] and 19% [range: 2–70%], respectively. Of the 23 patients in the oFE Group, three and one patients exhibited ventriculomegaly and cerebral infarction, respectively, in neuroimaging. Two patients had been born prematurely, with neonatal asphyxia in one of these cases. Ten patients had intellectual deficits of unknown cause. Mean SWI in the initial EEG record was 31% [range: 3–82%]. Two and one patients in the Panayiotopoulos and oFE Groups, respectively, did not take medication because they had seizures only rarely. Of the 22 patients in the Latent Group, EEG was recorded in 14 for reasons related to developmental behavioral disorders, in four for the investigation of brain functions associated with neurological/intellectual deficits, and in the other four for differential diagnosis related to epilepsy. This group's mean SWI in the initial EEG record was 13% [range: 2–43%]. Eighteen of 22 patients in the Latent Group did not take medication.

3.2. HFOs in the initial EEG record

Regarding HFOs in the initial EEG records, the number of ripples associated with spikes was significantly higher in the iCSWS Group (median 424.5) than in the BECTS, Panayiotopoulos, oFE, and Latent Groups (median 12, 0.5, 15, 0, respectively, with $p = 0.0021$, 0.0005, 0.0015, 0.0002, respectively), in the BECTS Group than in the Latent Group ($p = 0.0061$), and in the nCSWS Group (median 660) than in the oFE and Latent Groups ($p = 0.0226$ and $p = 0.0128$, respectively), as indicated in Fig. 2A. The number of ripples without spikes (median 0 in all the diagnostic groups) showed no statistically significant differences among the groups in any combination (Fig. 2B). The number of ripples without spikes (median across all patients: 0; mean: 0.1; range: 0–7) was smaller than the number of ripples coupled with spikes (median across all patients: 7; mean: 117.6; range: 0–878; $p < 0.0001$). Within each of the iCSWS, BECTS, Panayiotopoulos, and oFE Groups, likewise, the number of ripples without spikes was smaller than that coupled with spikes ($p < 0.01$). Regarding

Table 1
Patient groups.

Group/type of disorder	No. of patients [M/F]	SZ onset/initial EEG mean age [range]	Clinical diagnosis in detail	Etiology/neurological findings/neuroimaging[a]	No. of EEG records
1/iCSWS	10 [6/4]	4.6 y [2.6 y–9.1 y]/ 7.2 y [2.6 y–11.3 y]	CSWS (8), ABPE (1), LKS (1)	Initially no abnormality	50
2/nCSWS	4 [1/3]	2.5 y [1.5 y–3.3 y]/ 6.0 y [3.8 y–8.8 y]	CSWS (4)	Intellectual disability (3), CNS malformation (4), congenital CMV infection (1)	22
3/BECTS	19 [7/12]	7.0 y [2.5 y–11.3y]/ 8.5 y [3.8 y–12.2 y]	BECTS (19)	No abnormality	102
4/Panay.	16 [9/7]	4.5 y [2.3 y–8.3 y]/ 5.7 y [2.3 y–9.7 y]	Panayiotopoulos syndrome (16)	No abnormality	73
5/oFE	23 [18/5]	4.0 y [1.3 y–8.8 y]/ 5.6 y [2.7 y–11.0 y]	FE with structural brain pathology (6), FE with intellectual disability or developmental disorder of unknown etiology (12), FE with possible brain pathology (5)	Intellectual disability (10), perinatal abnormality (3), CNS malformation (4), cerebrovascular disorder (2)	156
6/Latent	22 [14/8]	NA/ 7.3 y [2.3 y–12.0 y]	Developmental disorder (12), headache (2), tics (2), somnambulism (1), intellectual disability (5)	Intellectual disability (5), congenital CMV infection (1), CNS malformation (1), Pierre-Robin syndrome (1)	78

(), number of the corresponding patients; M, male; F, female; y, year; SZ, seizure; EEG, electroencephalogram; CSWS, epileptic encephalopathy with continuous spike-and-wave during sleep; iCSWS, idiopathic CSWS; nCSWS, non-idiopathic CSWS; BECTS, benign epilepsy with centrotemporal spikes; Panay., Panayiotopoulos syndrome; oFE, other types of focal epilepsies; FS, febrile seizure; ABPE, atypical benign partial epilepsy; LKS, Landau-Kleffner syndrome; CMV, cytomegalovirus; CNS, central nerve system; NA, not applicable.

[a] Only patients with clarified etiology, neurological findings, and/or neuroimaging findings are indicated with duplication.

Table 2
CSWS Patients Idiopathic CSWS (iCSWS) Group.

Patient/gender	SWI (%) [specific diagnosis]	Age (Sz onset/Initial EEG/Last follow-up)	Seizure type	IQ (age at examination)	Development before epilepsy and neurological findings	Lesion and etiology
1/M	95	3 y/8.3 y/12.8 y	Nocturnal vomiting and subsequent L HSz	80 (8.4 y), 88 (12.4 y)	ADHD	Not found
2/F	87[a]	6 y/8.6 y/9.3 y	Nocturnal sylvian Sz and GSz	Normal (short follow-up period)	Normal	Not found
3/F	87 [ABPE]	7.3 y/11.3 y/15.9 y	GSz during drowsiness, absence	96 (15.9 y)	Normal	Not found
4/F	93	6.6 y/7.6 y/17.8 y	Nocturnal FS with vomiting and eye-deviation evolving into R HSz	92 (7.6 y), 83 (10.5 y)	Normal	Not found
5/M	92	4.8 y/6.6 y/17.8 y	Nocturnal GSz with eye-deviation including two episodes of SE	76 (8.8 y), 70 (11.8 y)	ADHD	Not found
6/F	92[b]	2.6 y/2.6 y/7.6 y	FS with vomiting on arousal evolving into GSz	89 (DQ: 2.6 y), 62 (4.8 y)	ADHD	Not found
7/M	98	6.3 y/9.2 y/16.7 y	Nocturnal R HSz, atypical absence	65 (10.5 y), 55 (12.5 y), 59 (16.7 y)	Normal	Not found
8/M	92[a]	3.3 y/4.9 y/ 7.4 y	Nocturnal sylvian Sz, atypical absence	102 (4.9 y), 81 (7.4 y)	ADHD	Not found
9/M	93[a]	5.4 y/7.3 y/7.5 y	Nocturnal sylvian Sz and GSz, absence, negative myoclonus	82 (7 y), 56 (7.3 y)	ASD, ADHD	Not found
10/M	93[a] [LKS]	9.1 y/10.2 y/12.7 y	Nocturnal FS and GSz, aphasia	69 (10.2 y), 99 (10.8 y), 102 (12.7 y)	Normal	Not found

Non-idiopathic CSWS (nCSWS) Group

Patient/gender	SWI (%)	Age (Sz onset/initial EEG/last follow-up)	Seizure type	IQ (age at examination)	Development and neurological findings	Lesion and etiology
1/F	98	2.9 y/3.8 y/9 y	L HSz during drowsiness, absence	51 (4.1 y), 47 (4.9 y), 36 (5.6 y), 27 (6.3 y)	Intellectual disability	Ectopic gray matter
2/F	100	1.5 y/6.6 y/12.4 y	Nocturnal FS with eye-deviation and GSz, absence	82 (6 y), 53 (8.8 y), 66 (11.8 y)	L hemiparesis	R polymicrogyria
3/M	100	2.1 y/8.8 y/15.5 y	Nocturnal right HSz and GSz including an episode of SE	97 (6 y), 61 (9.2 y)	Paraplegia, bladder and rectal disturbance	Spinal bifida cystica, hydrocephalus
4/F	97	3.3 y/4.9 y/7.8 y	Atypical absence	40 (DQ: 5 y), 33 (DQ: 7.7 y), 30 (7.8 y)	R paresis, intellectual disability	L dominant brain malformation due to congenital CMV infection

F, female; M, male; y, year; L, left; R, right; Sz; seizure; HSz, hemiseizure; GSz; generalized Sz; FS, focal Sz; SE, status epilepticus; EEG, electroencephalogram; MRI, magnetic resonance imaging; CSWS, epileptic encephalopathy with continuous spike-and-wave during sleep; SWI, spike-wave index in the initially recorded sleep EEG; BECTS, benign epilepsy with centrotemporal spikes; ABPE, atypical benign partial epilepsy; LKS, Landau-Kleffner syndrome; ASD, autism spectrum disorder; ADHD, attention-deficit/hyperactivity disorder; CMV, cytomegalovirus; IQ, Intelligent quotient; DQ, developmental quotient

[a] Evolved from BECTS.

[b] Evolved from Panayiotopoulos syndrome.

the localization of a total of 120 ripples without spikes, 64 (53.3%) were over the bilateral occipital, midline central, and midline parietal regions. The number of spikes was significantly higher in the iCSWS Group (median 73) than in the BECTS, Panayiotopoulos, oFE and Latent Groups (median 43, 17, 40, 13.5, respectively, with $p = 0.0132$, 0.0041, 0.0387, 0.0002, respectively), in the BECTS Group than in the Latent Group ($p = 0.0017$), and in the nCSWS Group (median 98.5) than in the BECTS, Panayiotopoulos and Latent Groups ($p = 0.0408$, 0.0451, 0.0242, respectively) (Fig. 2C).

Ratio of ripples per spike in the initial EEG was higher in the iCSWS Group (median 5.73) than in the BECTS, Panayiotopoulos, oFE and Latent Groups (median 0.39, 0.02, 0.35, 0, respectively, with $p = 0.005$, 0.0006, 0.0032, 0.0002, respectively), in the BECTS Group than in the Latent Group ($p = 0.0079$), and in the nCSWS (median 5.74) and oFE Groups than in the Latent Group ($p = 0.0344$ and 0.0365, respectively) (Fig. 2D). Regarding the frequency and duration of ripples, no statistically significant differences were found in any combination of groups (Fig. 2E, F, respectively).

3.3. Age-dependent changes in HFOs and their relationship to clinical features

As indicated in Fig. 3, which shows age-dependent changes in the parameters of ripples and spikes in each group, the number of ripples associated with spikes and the ratio of ripples per spike tended to be higher at younger ages, particularly in the iCSWS and nCSWS Groups, and to vanish in preadolescence/adolescence in all of the diagnostic groups (Fig. 3A, D). The number of spikes tended to lag behind that of ripples associated with spikes in an age-dependent pattern of decline (Fig. 3C). Regarding the number of ripples without spikes and the frequencies and durations of ripples, there were no apparent age-dependent changes in any group (Fig. 3B, E, F, respectively).

The number of spikes and that of ripples with and without spikes were compared between childhood < 10 years of age (mean 6.7 years) and preadolescence/adolescence (mean 13.1 years) in 36 patients (4, 2, 10, 3, 12, and 5 in the iCSWS, nCSWS, BECTS, Panayiotopoulos, oFE, and Latent Groups, respectively). The number of spikes was significantly higher during childhood than during preadolescence/adolescence (median 42 vs. 3, $p < 0.0001$; $p = 0.0046$ regarding the combined group of BECTS and Panayiotopoulos syndrome, and differences not significant regarding the other groups probably due to small number of patients), as was the number of ripples with spikes (median 27.5 vs. 0, $p < 0.0001$; $p = 0.0244$ regarding the combined group of BECTS and Panayiotopoulos syndrome, $p = 0.0020$ regarding the oFE Group, and differences not significant regarding the other groups). The number of ripples without spikes was not different between the two age ranges (median 0 vs. 0, $p = 1.0$). Ratio of ripples per spike was compared between the two age ranges (mean 6.6 and 12.6 years, respectively) in 20 patients (4, 1, 3, 3, 5, and 4, respectively) who

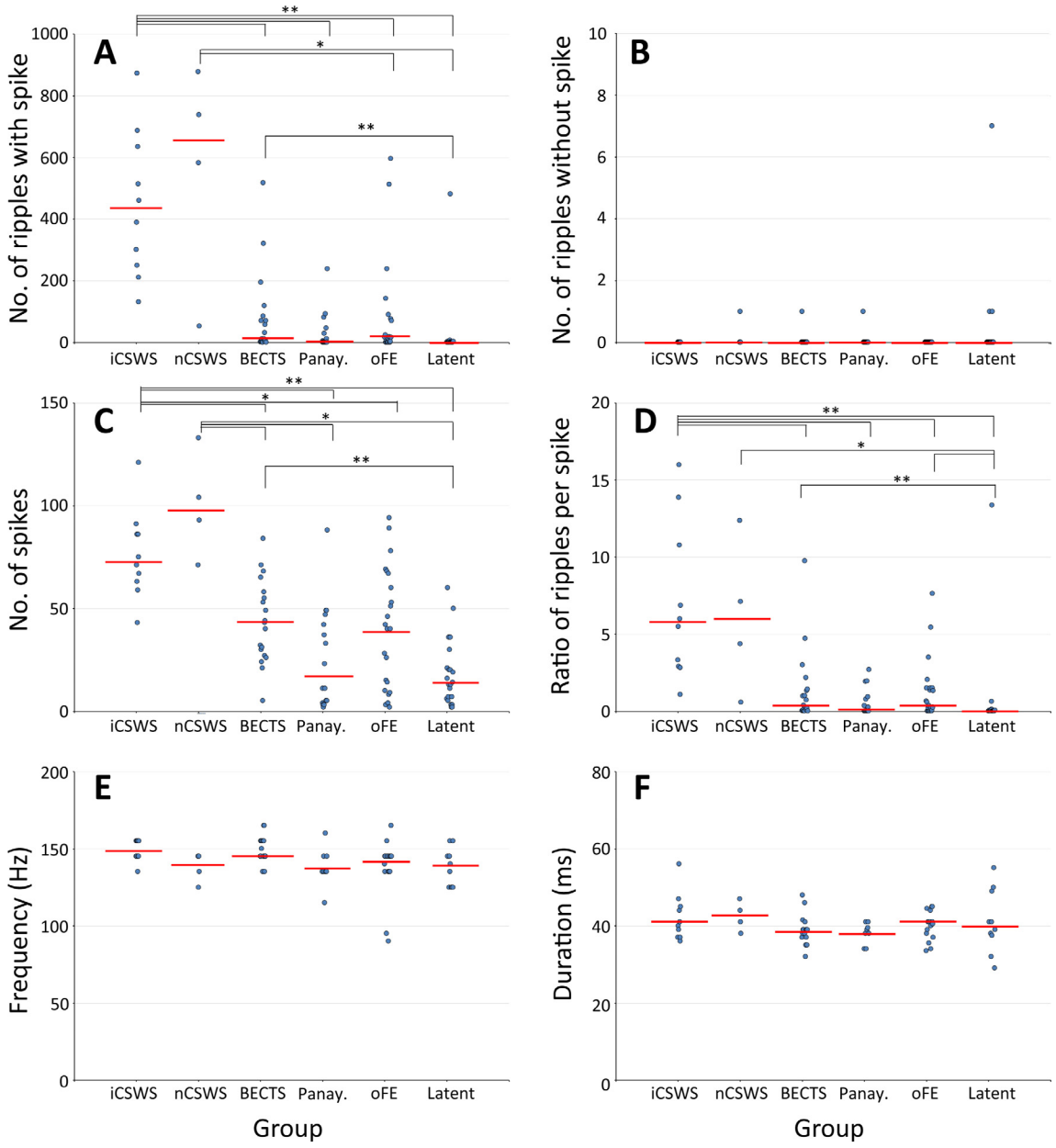

Fig. 2. Distribution of parameters of ripples and spikes in the initial EEG record across the patient groups. (A) The number of ripples associated with spikes was significantly higher in the iCSWS Group than in any other group except for the nCSWS Group (**$p < 0.01$), in the BECTS Group than in the Latent Group (**$p < 0.01$), and in the nCSWS Group than in the oFE and Latent Groups (*$p < 0.05$) as revealed by the Steel–Dwass test. (B) The number of ripples without spikes was very small with no statistically significant differences in any combination of groups. (C) The number of spikes was significantly higher in the iCSWS Group than in either the Panayiotopoulos (Panay.) and Latent Groups (**$p < 0.01$) or the BECTS and oEF Groups (*$p < 0.05$), in the BECTS Group than in the Latent Group (**$p < 0.01$), and in the nCSWS Group than in the BECTS, Panayiotopoulos and Latent Groups (*$p < 0.05$). D: Ratio of ripples per spike was higher in the iCSWS Group than in any other group except for the nCSWS Group (**$p < 0.01$), in the BECTS Group than in the Latent Group (**$p < 0.01$), and in the nCSWS and oFE Groups than in the Latent Group (*$p < 0.05$). Regarding the frequency (E) and duration (F) of ripples, no statistically significant differences were found in any combination of groups. Horizontal bars indicate median values.

had spikes during both age ranges and was found to be significantly higher during childhood (median 1.495 vs. 0.008, $p = 0.0155$; differences not significant in the individual groups probably due to small number of patients). The frequency and duration of ripples were compared between the two age ranges (mean 6.3 and 11.9 years, respectively) in 8 patients (2, 1, 1, 2, 2, and 0, respectively) who had ripples during both age ranges, and there were no significant differences (median 145 Hz vs. 135 Hz, $p = 0.3750$ and 41.0 ms vs. 38.8 ms, $p = 0.0547$, respectively).

Age-dependent changes in the ratio of ripples per spike in individual patients in each group are shown in Fig. 4. Patients exhibiting a decline in IQ/DQ, no remarkable changes in IQ, and transient language disorder with subsequent recovery of IQ are indicated in red, blue, and green, respectively, in the iCSWS and nCSWS Groups (Fig. 4A, B, respectively). In the iCSWS Group, it is conceivable that patients who had high ratios of ripples per spike at a young age were more likely to present with intellectual decline. In contrast, patients who exhibited ripples during school age showed language recovery or no remarkable intellectual disturbances.

In the BECTS and Panayiotopoulos Groups, some patients showed unusually high ratios of ripples per spike (Fig. 4C, D, respectively). Such changes, however, were only transient and rather exceptional. These children showed no intellectual decline. In the oFE Group, ratio of ripples per spike was not high and varied

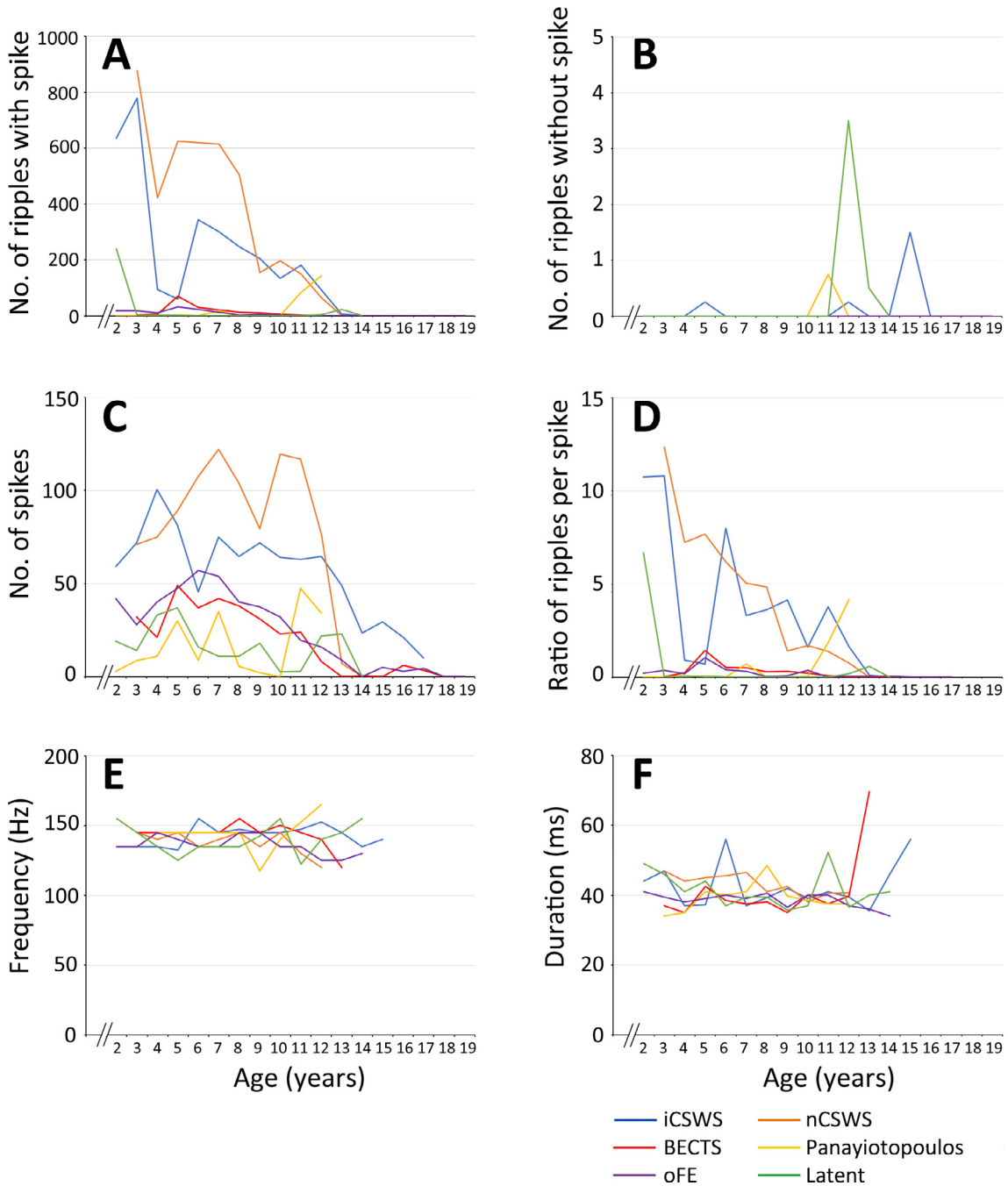

Fig. 3. Age-dependent changes in the parameters of ripples and spikes in each group. Group-wise median values of number of ripples associated with spikes (A), ripples without spikes (B), number of spikes (C), ratio of ripples per spike (D), frequency of ripples (E), and duration of ripples (F) in each group are indicated against age. Median of patient-wise median value in the group is shown with respect to frequency and duration of ripples. The number of ripples with spikes and ratio of ripples per spike tended to be higher in the younger age range, particularly in the iCSWS and nCSWS Groups, and to vanish in preadolescence/adolescence. The number of spikes tended to lag behind that of ripples with spikes in an age-dependent pattern of decline. Regarding the number of ripples without spike, frequencies and durations of ripples, there were no apparent age-dependent changes in any group. Patients were under medical treatment when the EEG data were recorded.

from patient to patient, which was indicative of the heterogenicity of this group (Fig. 4E). A patient in the Latent Group who exhibited a high ratio of ripples per spike at a young age had intellectual deficits with severe autistic behavior disorders (Fig. 4F).

4. Discussion

HFOs and FOs in scalp EEGs recorded from children have been shown to be a useful clinical marker for epilepsy severity in recent years (Kobayashi et al., 2015; van Klink et al., 2016). This is the first study to directly compare the amount of ripples generated in terms of total number of occurrences and ratio of ripples per spike and the characteristics of ripple oscillations among various types of pediatric epilepsies with the common characteristics of sleep-activated spikes in scalp EEG by semi-automatic detection with visual confirmation. In this study, we gained insights into the relationship between ripple incidence and clinical features, including age-dependent changes in patients with various types of epilepsies.

The CSWS EEG pattern is related to an epileptic encephalopathy of childhood requiring prompt diagnosis and treatment to improve

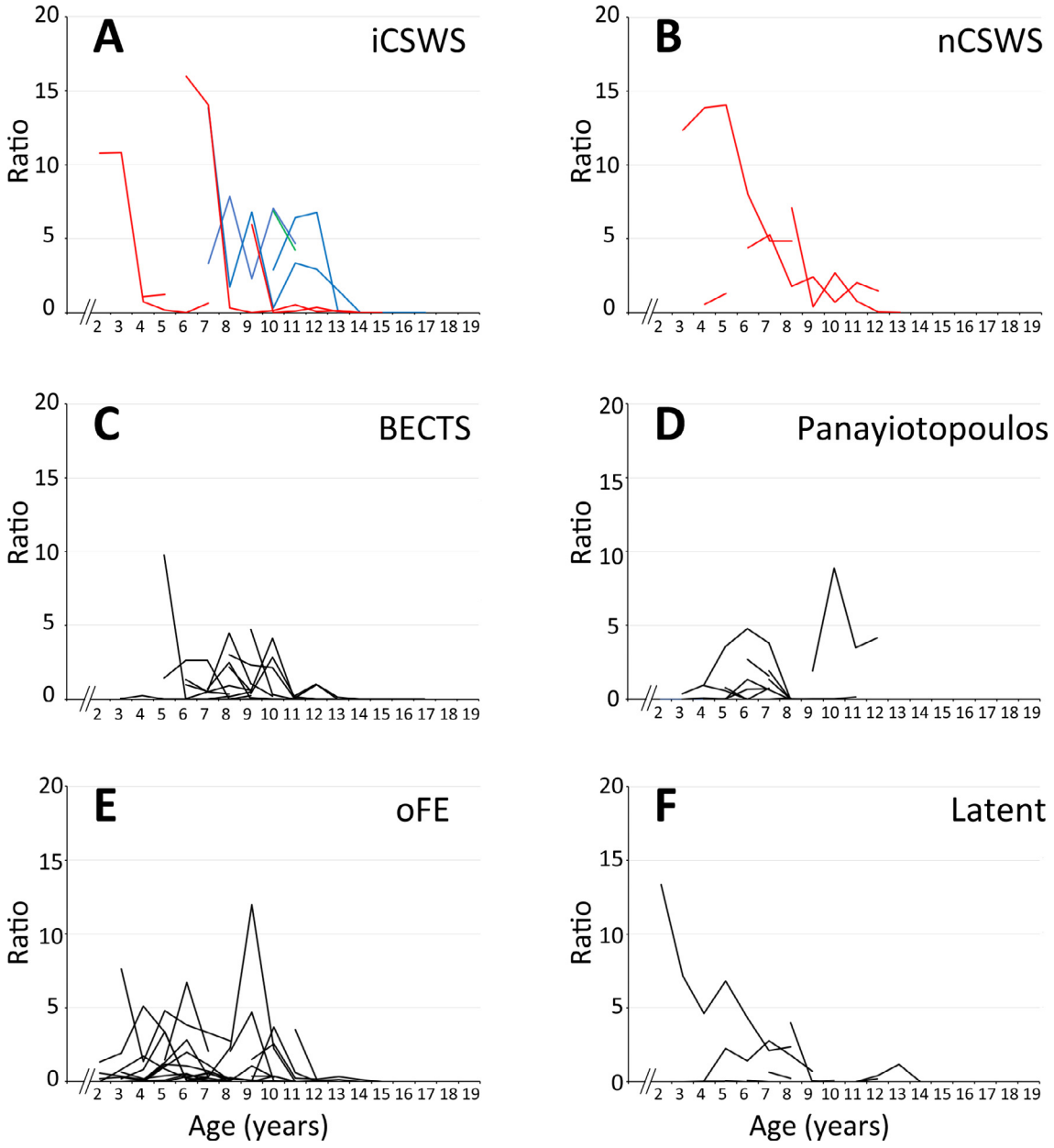

Fig. 4. Age-dependent changes in the ratio of ripples per spike in individual patients in each group. Ratio of ripples per spike is indicated against age in the iCSWS (A), nCSWS (B), BECTS (C), Panayiotopoulos (D), oFE (E), and Latent (F) Groups. Patients exhibiting a decline in IQ/DQ, no remarkable changes in IQ, and transient language disorder with subsequent recovery of IQ are shown in red, blue, and green, respectively, in the iCSWS and nCSWS Groups.

cognitive outcome. Occasionally, idiopathic focal epilepsies in childhood such as BECTS and Panayiotopoulos syndrome develop into epileptic encephalopathy with CSWS during follow-up. In our previous studies, more ripples appeared in the EEG data of CSWS patients than in that of patients with focal spikes without statistical comparison (Kobayashi et al., 2010, 2011). In the present study, we found that the abundant ripples in the initial EEG record resulted from the combination of the high number of spikes and the high ratio of ripples per spike in the iCSWS and nCSWS Groups. With respect to age-dependent changes in ripples and spikes, the generation of ripples associated with spikes was intense at younger ages. Their age-dependent dissipation was due not only to a reduction in the number of spikes but also to a reduction in the ratio of ripples per spike, suggesting that the pathogenicity of each individual spike might lessen according to age. Taken together, our findings suggest that a large proportion of pathological HFOs may be related to functional disruption of the physiological brain networks, which are thought to work through physiological high-frequency activity, and that this disruption may be linked to epileptic encephalopathy in childhood. In addition, an abundance of ripples prior to 10 years of age appeared to be more closely related to persistent disturbance of intelligence than an abundance of ripples during preadolescence/adolescence in the iCSWS Group. In a previous study, De Giorgis et al. (2017) found that children with an early onset of CSWS were intellectually more vulnerable than those with a late onset. Cognitive outcome may depend on treatment response to EEG abnormality and seizures (Liukkonen et al., 2010), but this issue is still under discussion (Japaridze et al., 2014; Fernández et al., 2012). The parameters describing the frequency and duration of ripples did not show age-dependent changes in the present study, and we should further investigate whether the nature of individual ripples really remains

identical rather than being age-dependent. The effects of different etiologies on the age-dependent changes in ripples could not be thoroughly investigated because only a small number of patients were examined during both childhood <10 years of age and preadolescence/adolescence. This issue should be clarified in future studies.

We reported that epileptogenicity related to rolandic spikes is relevant to the associated ripples (Kobayashi et al., 2011; Shibata et al., 2016). van Klink et al. (2016) reported that the presence of ripples associated with rolandic spikes correlated with seizure frequency and suggested that scalp ripples reflect the severity of epilepsy. In the present study, the detection of a greater number of ripples in the BECTS Group than in the Latent Group confirmed this suggestion, as did the stormy generation of ripples in association with severe epilepsies characterized by the CSWS pattern. The frequency and duration of ripples did not appear to have a direct relationship to epilepsy severity.

In the Panayiotopoulos Group, there were some patients who had no or only minimal ripples despite persisting epileptic discharges for a long period of time, although one patient exceptionally exhibited ripples during preadolescence/adolescence. Shibata et al. (2016) reported that the proportion of spike-associated high-frequency activity was lower in Panayiotopoulos syndrome than in BECTS, and indicated that sleep-activated spikes in Panayiotopoulos syndrome may be less relevant to epileptogenicity than rolandic spikes are in BECTS.

There were some patients with a rather large number of ripples in the oFE and Latent Groups in the present study, and such ripples may reflect etiology. The generation of ripples may be also affected by other factors including treatment, and might have a relationship with associated neurodevelopmental disorders. These questions, however, remain open and should be addressed in future studies.

Although a visual review by experienced electroencephalographers is the gold standard for HFO identification, the performance of the semi-automatic detection tool for HFOs/FOs that we used in this study has already been confirmed (von Ellenrieder et al., 2012). It provides the advantage of an objective detection of HFOs, in addition to a reduction in analysis time. We admit, however, that the usage of a cut-off threshold of power to detect ripples presents an intrinsic problem, particularly in EEG data with abundant HFOs that might affect the threshold. There were apparent oscillatory activities that were not picked up by the detection tool, probably because of insufficient power increase. The low number of ripples without spikes might be a result of this methodological issue. In the present study, ripples on different scalp electrodes were counted separately because they did appear largely asynchronous, as exemplified in Fig. 1A. Theoretically, ripples on scalp electrodes that are several centimeters apart should not have an identical cortical origin because the extent of cortical generators is indicated to be small, with an area of approximately 1 cm^2 (von Ellenrieder et al., 2014; Zelmann et al., 2014).

In the present study, the number of ripples unassociated with spikes was very small with no statistically significant differences in any combination of diagnostic groups and no age-dependent changes. As such, we suggest that ripples without spikes may be largely irrelevant to epileptogenicity. Mooij et al. (2017) reported the presence of ripples in spike-free EEGs recorded from children with and without epilepsy, and suggested that these ripples were physiological. Physiological ripples were reportedly predominant over the midline central and parietal regions but were also observed in the occipital lobes (Alkawadri et al., 2014): these findings are in agreement with the scalp distribution of ripples without spikes found in the present study. Therefore, it may be justifiable to focus only on ripples associated with spikes in order to exclude possibly non-epileptic ripples.

The limitations of our study included our inability to differentiate the effects of increasing age on ripple dissipation from those of medication because all CSWS patients were medically treated. We analyzed EEG data that were recorded mostly under treatment in all diagnostic groups. In this regard, medication might be related to the ripple decrease associated with increasing age. In addition, our methods of visual correction of ripples might bias the detection towards higher-amplitude ripple events and cause us to miss some lower-amplitude events. There were some ripple oscillations that could be barely observed without filtration of EEG data (as exemplified in Fig. 1B), but most ripples embedded in high-amplitude spikes were hardly visible without filtration. Therefore, we cannot deny the possibility that our study includes some false-ripples that were in fact contaminated muscle activity or filtering artifacts. The high-pass filtering of a higher-amplitude, very sharply-contoured wave might have resulted in the apparent formation of HFOs with four or more cycles. Such false-HFOs are not always differentiable from true HFOs (Motoi et al., 2018). However, the developers of the present program (von Ellenrieder et al., 2012) have made it unlikely that the detected events result from the filtering of sharp transients. False-HFOs produced by filter effects have been suggested to be nearly as useful as true HFOs in the discrimination of the seizure onset zone (SOZ) from non-SOZ (Burnos et al., 2016). The issue of false-HFOs remains to be solved, but we hope to completely differentiate between true and false-ripples in scalp EEG data to clarify their meanings in future.

The number of detected ripples may depend on the methodologies used. The number of ripples in the present study employing the semi-automatic detection tool may not be directly compared to that in other reports relying on visual review (van Klink et al., 2016). When time-frequency analysis is used for the identification of ripples in addition to visual review, their detection may be reduced because the spectral power of ripples is generally low, and hence blobs of ripples tend to be buried in background activity power. Despite all these technical issues, the study of non-invasively recorded HFOs should enhance our understanding of epileptogenicity, as it allowed us in this study to statistically confirm, for the first time, the characteristics of massive generation of ripples in pediatric patients with epileptic encephalopathy with CSWS. Recently, even the detection of fast ripples was reported from the scalp EEG of infants with tuberous sclerosis complex (Bernardo et al., 2018). We could not investigate fast ripples because the EEG sampling rate was 500 Hz. Further studies are needed to clarify the relationship between pathological scalp HFOs and functional disruption of the brain networks in epileptic encephalopathy involving a large number of pediatric patients and advanced EEG recording systems with high-density electrodes and a high sampling rate.

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Declaration of Competing Interest

None of the authors have potential conflicts of interest to be disclosed.

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