Accurate differentiation between physiological and pathological ripples recorded with scalp-EEG

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ARTICLE INFO

HIGHLIGHTS

- Physiological ripples start after co-occurring vertex waves, while pathological ripples start around the start of co-occurring IEDs.
- Scalp-EEG recorded physiological ripples have longer durations, lower frequencies, and lower amplitudes than pathological ripples.
- Pathological ripples in EEGs of children with idiopathic epilepsy may differ from ripples in EEGs of children with symptomatic epilepsy.

ABSTRACT

Objective: To compare scalp-EEG recorded physiological ripples co-occurring with vertex waves to pathological ripples co-occurring with interictal epileptiform discharges (IEDs).
Methods: We marked ripples in sleep EEGs of children. We compared the start of ripples to vertex wave- or IED-start, and duration, frequency, and root mean square (RMS) amplitude of physiological and pathological ripples using multilevel modeling. Ripples were classified as physiological or pathological using linear discriminant analysis.
Results: We included 40 children with and without epilepsy. Ripples started ($\chi^2(1)$ = 38.59, p < 0.001) later if they co-occurred with vertex waves (108.2 ms after vertex wave-start) than if they co-occurred with IEDs (4.3 ms after IED-start). Physiological ripples had longer durations (75.7 ms vs 53.0 ms), lower frequencies (98.3 Hz vs 130.6 Hz), and lower RMS amplitudes (0.9 μV vs 1.8 μV, all p < 0.001) than pathological ripples. Ripples could be classified as physiological or pathological with 98 % accuracy. Ripples recorded in children with idiopathic or symptomatic epilepsy seemed to form two subgroups of pathological ripples.
Conclusions: Ripples co-occurring with vertex waves or IEDs have different characteristics and can be differentiated as physiological or pathological with high accuracy.
Significance: This is the first study that compares physiological and pathological ripples recorded with scalp EEG.

1. Introduction

Ripples are physiological or pathological brain oscillations with frequencies between 80–250 Hz (Kane et al. 2017). Physiological ripples are related to cognitive functions. They were, for example, shown to be involved in memory retrieval and consolidation (Rothschild et al., 2017; Vaz et al., 2019). Pathological ripples are

considered biomarkers for epilepsy (Frauscher et al. 2017; Thomschewski et al. 2019; Jacobs and Zijlmans 2020). Considering this difference in neurophysiological significance, it is important to be able to recognize physiological and pathological ripples. To this end, several studies have compared duration, frequency, and amplitude of presumed physiological and pathological ripples (Nagasawa et al. 2012; Wang et al. 2013; Matsumoto et al. 2013; Alkawadri et al. 2014; Malinowska et al. 2015; Bruder et al. 2017; von Ellenrieder et al. 2016; Pail et al. 2017; Cimbalnik et al. 2018; Charupanit et al. 2020; Migliorelli et al. 2021). The results are contradictory (see Supplementary Table 1 for an overview of the results per study), especially for duration and frequency. Differences between amplitudes of presumed physiological and pathological ripples seem to be more consistent and suggest that physiological ripples may have lower amplitudes than pathological ripples. All studies listed in Supplementary Table 1 were performed on intracranial EEG recordings.

The aim of this study is to compare physiological and pathological ripples recorded with scalp EEG. We have previously shown that scalp EEG-recorded physiological ripples often co-occur with sleep-specific transients (Mooij et al. 2018). For this study, we selected ripples co-occurring with vertex waves as the physiological ripples to compare to pathological ripples co-occurring with interictal epileptiform discharges (IEDs: spikes [20–70 ms] or sharp waves [70–200 ms]). We chose vertex wave-ripples for two reasons. First, vertex waves were the sleep-specific transient with which physiological ripples co-occurred most often and in most children (Mooij et al. 2018). Second, physiological vertex waves resemble pathological IEDs in several ways: they are both multiphasic, short, sharp transients that stand out from the background EEG (Berry et al. 2012; Kane et al. 2017). Pathological ripples co-occurring with spikes were found to start before the start or during the first phase of the spike (van Klink et al. 2016; Cai et al. 2021; Guth et al. 2021), suggesting that the ripples are not generated by spikes. We reported that 74 % of physiological ripples co-occurred with sleep-specific transients and speculated that sleep-specific transients may facilitate physiological ripple generation. The start of physiological ripples relative to the start of co-occurring sleep specific transients was not investigated in that study (Mooij et al. 2018).

We therefore addressed two questions: 1) What is the timing of the start of scalp-EEG recorded physiological and pathological ripples relative to the start of co-occurring vertex waves or IEDs? 2) Do scalp-EEG recorded physiological and pathological ripples have the same or different duration, frequency, and amplitude?

2. Methods

2.1. Participants

We retrospectively studied EEGs of children (17 years or younger) who had visited our outpatient first seizure clinic between May 2013 and May 2016 because they were suspected of having had one or more (first) possible seizure(s). We reviewed EEGs from all children who had an EEG recorded at high sampling rate (2048 Hz) with a minimum of 10 minutes of sleep recording. A specialized pediatric neurologist determined the eventual diagnosis of included children based on all information available in the clinical files until the end of follow-up (April 2021). The Medical Research Ethics Committee of the University Medical Center Utrecht judged that the Dutch Medical Research Involving Human Subjects Act did not apply, and written consent was not required, provided that data were coded and handled anonymously.

2.2. EEG recordings

Scalp EEGs were recorded with Micromed Smart Acquisition Module (SAM) and with SD PLUS FLEXI acquisition system (Micromed, Treviso, Italy). Data were sampled at 2048 Hz, low pass anti-alias filter at acquisition was 900 Hz for SAM and 553 Hz for FLEXI. We used conventional 10 mm Ag-AgCl electrodes that were placed according to the international 10–20 system.

2.3. Marking ripples

We marked ripples that were visible in both average and bipolar montages in Stellate Harmony (Montreal, Canada) whenever the signal-to-noise ratio of the sleep EEG allowed proper judgement. The signal was filtered between 80 Hz (finite impulse response (FIR) high-pass filter of order 63) and 250 Hz (FIR low-pass filter of order 63), amplitude scale was set to 1 μV per mm, time scale was 0.9 seconds per page. This was the setting that showed each recorded data sample for the size of the screen. Ripples were defined as four or more oscillations that clearly stood out from the background EEG signal. If background low-amplitude ripple band activity gradually built up to visually recognizable ripple oscillations, we started marking when the oscillations stood out clearly from the overall background activity of the channel and stopped marking when this was no longer the case. We marked ripples in all channels in which they occurred. If ripples occurred in a cluster, i.e., their duration overlapped with the duration of ripples occurring on other channels, we selected the ripple that stood out most clearly from the background in the average montage as the dominant ripple. We chose the average montage because there was generally less noise in the EEG signal viewed in the average montage than viewed in the bipolar montage. Ripples were marked by AM and checked by MZ.

2.4. Marking the start of vertex waves and IEDs'

We viewed the low frequency EEG at the time of a ripple to see if there was a co-occurring vertex wave or IED. These events were identified following standard definitions (Berry et al. 2012; Kane et al. 2017). In case of doubt (for example: a broad vertex wave or a small K-complex), consensus was reached by two reviewers (AM & CF). The EEG signal was filtered between 0.5 (infinite impulse response filter (IIR) of order 3) and 250 Hz (FIR low-pass filter of order 63). Time scale was 10 seconds per page (on a 15.4-inch screen), amplitude scale varied between 10 to 30 μV/mm depending on the amplitude of the signal.

We marked the start of co-occurring vertex waves and IEDs (Fig. 1) for ripples marked in average montage. If ripples occurred in clusters, we marked the start of the vertex wave or IED that co-occurred with the dominant ripple marked in the average montage. We would know that there was a co-occurring ripple, but we made the ripple itself invisible when marking the start of the vertex wave or IED, to avoid being biased by the timing of the ripple. The start marker of the vertex wave or IED was placed at the start of the first downward or upward deflection. Vertex waves and IEDs were marked by AM and checked by CF.

2.5. Calculating ripple characteristics

We used solitary ripples and dominant ripples marked in the average montage for the analysis. We calculated for each ripple: 1) The duration (in ms) from start to stop of the ripple markings. 2) The frequency (in Hz). We calculated ripple frequency as 1/duration of one oscillation. Duration of one oscillation was calculated by dividing the duration of the ripple by the total number of oscillations. The total number of oscillations was calculated as the num-
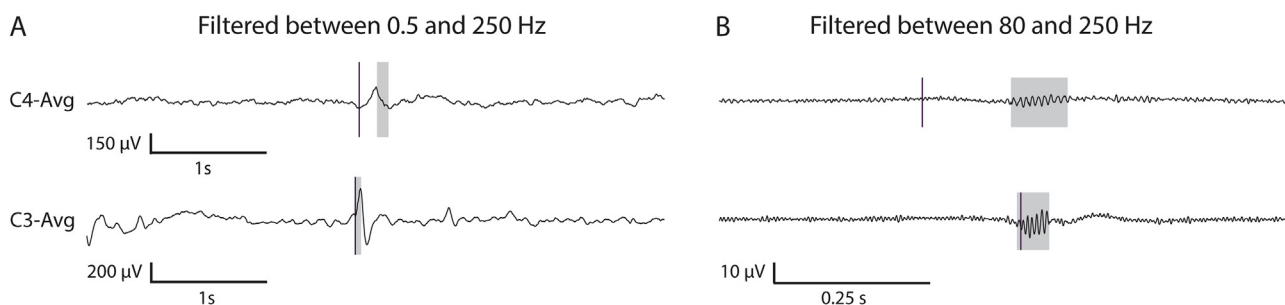
A   Filtered between 0.5 and 250 Hz   B   Filtered between 80 and 250 Hz

Fig. 1. Example of ripples (in B) co-occurring with a vertex wave (first trace in A) and an interictal epileptic discharge (spike, second trace in A) marked in average (Avg) montage. Time scale was 5 seconds for traces in A, amplitude scale was 15 μV/mm for the first trace, and 20 μV/mm for the second trace in A. Time scale was 1 second and amplitude scale was 1 μV/mm for both traces in B. A vertical line marks the start of the vertex wave and the spike. Gray markings and vertical lines in A correspond to the same markings in B. The physiological ripple occurs after the start of the vertex wave, while the pathological ripple starts at the same time as the spike.

ber of zero crossings of the filtered ripple band within the duration of the marked ripple divided by two. 3) The root mean square (RMS) amplitude (in μV).

2.6. Multilevel analyses

This study contains nested data, namely several children with multiple observations per child. We used a multilevel analysis to accommodate for this dependency in the data. Only ripples from EEGs with at least three solitary or dominant physiological or pathological ripples were included in the analysis. Statistical analyses were done in R 4.1.2 (R Core Team, 2021).

We first fitted a model with random intercepts to quantify the amount of dependency in the data. We then added the type of co-occurring event (vertex wave or IED) as fixed effect to the model to determine if there was a difference in characteristics of ripples co-occurring with these events while controlling for dependency in the data. Differences between models were tested using a difference in deviance (-2^* Log Likelihood) test, which is chi-square distributed. The used α was set at 0.05. We also calculated Cohen's d (including a correction factor for sample size at the patient level to prevent inflated effect sizes) and the explained between subject variance as measures of the effect size.

We performed the same analysis for ripple start relative to vertex wave or IED start and for ripple duration, frequency, and RMS amplitude. We also performed this analysis for the number of oscillations, because the interpretation of the results of ripple duration and frequency depends on the number of oscillations of the ripples. For example, if one type of ripple has a shorter duration than the other, this may be because of a difference in the number of oscillations, because of a difference in frequency (with the same number of oscillations, ripples with higher frequency have a shorter duration), or both.

2.7. Classification of physiological and pathological ripples

We applied linear discriminant analysis to test if potential differences between ripple characteristics could be used to classify them as physiological or pathological ripples. Presented outcomes are the average over 10 runs of 5-fold cross validation, each time using a randomly selected subset of 75 % as training set, and the remaining ripples as testing set. We calculated sensitivity, specificity, positive predictive value, negative predictive value, and accuracy to evaluate the performance of the classification.

2.8. Ripples without co-occurring events

Our aim was to describe and compare characteristics of physiological and pathological ripples recorded with scalp EEG. We

defined ripples as pathological if they co-occurred with IEDs; the co-occurrence of vertex waves was used as the physiological equivalent of IEDs. Limiting the doubt about the physiological or pathological nature of the ripples is important when describing their characteristics. However, the real challenge in ripple marking lies in discriminating ripples without co-occurring physiological or pathological events. As an additional step, we selected ripples without an IED, or a vertex wave or other physiological sleep-specific transient, occurring on the same or another channel at the time of the ripple. This strict criterium ensured that we kept only the ripples that occurred without any simultaneously occurring event in the EEG that might help classify them as physiological or pathological. Ripples occurring in normal EEGs were considered physiological. Ripples occurring in EEGs that contained IEDs were labeled in line with previously marked events in that EEG, i.e., taking into account ripple appearance and their occurrence in 'normal' channels or channels containing IEDs. We compared the characteristics of the ripples without co-occurring event to the characteristics of ripples with co-occurring events using the linear discriminant model obtained in the previous subsection, i.e., the model based on ripples with co-occurring events.

3. Results

3.1. Children, EEGs, and diagnoses

There were 345 EEGs recorded in this cohort during May 2013 and May 2016, 76 of which contained sleep (all daytime recordings). Thirty-six EEGs were excluded for the following reasons: quality of the EEG not sufficient for ripple marking or not enough sleep (4 EEGs); no physiological or pathological ripples (7 normal EEGs and 6 EEGs containing IEDs); ripples co-occurring with other sleep-specific transients, such as sleep spindles or K-complexes, but not with vertex waves (14 EEGs, 7 normal EEGs and 7 containing IEDs). In addition, two EEGs were excluded because reviewers could not determine the start of the co-occurring vertex wave (1 ripple in 1 EEG) or it was not clear if the co-occurring event was an IED or an aberrant K-complex (1 ripple cluster in 1 EEG), and three normal EEGs were excluded because parents gave no permission for inclusion in a previous study.

We included EEGs from 40 children (Table 1). Twenty-two children had a normal EEG with ripples co-occurring with vertex waves (1–22), and 18 children (23–40) had an EEG showing IEDs: eight (23–30) with only ripples co-occurring with vertex waves, eight (33–40) with only ripples co-occurring with IEDs, and two (31–32) containing ripples co-occurring with both types of sharp EEG transient. Three EEGs (38–40) contained ripples co-occurring with (poly)spikes. It was difficult to mark an unequivocal start of the polyspikes. These ripples were therefore included in the analy-

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Table 1
Characteristics of included children.

	#	M/F	Age at EEG	Follow-up	MRI	Diagnosis	Medication	R + VW	R + IED	R −
Normal EEG, no IED present	1	M	1y6m	7y5m	Normal	None	no	6		6
	2	F	5y0m	6y11m	x	None	no	10		9
	3	M	8y11m	5y11m	Normal	None	no	8		0
	4	M	1y0m	5y9m	x	None	no	1		0
	5	M	5y1m	7y5m	x	Possibly sleep disorder	no	13		3
	6	F	3y8m	5y7m	Underdevelopment of the caudal vermis, absence of right internal carotid artery	PHACE syndrome	no	1		0
	7	M	8y6m	7y3m	Pylocytic astrocytoma in mesencephalon and bilateral thalamus	ADHD	methyl-phenidate	1		0
	8	M	8y10m	6y9m	Normal	Tic syndrome and autism spectrum disorder	no	6		14
	9	M	2y4m	7y7m	Normal	Autism	no	5		0
	10	M	5y2m	6y2m	Right frontal developmental venous analomy, otherwise normal	Autism and chromosomal microduplication syndrome	no	4		4
	11	F	7y5m	6y8m	Normal	Migraine, CACNA1A deficiency	no	3		5
	12	M	3y8m	7y6m	Normal	Glut 1 deficiency syndrome	no	4		0
	13	M	5y5m	5y2m	Probably old lesion in left cerebellar hemisphere	No diagnosis, epilepsy highly unlikely	no	3		1
	14	M	3y8m	7y2m	Normal	No diagnosis, epilepsy highly unlikely	no	7		8
	15	M	5y10m	5y9m	Normal	No diagnosis, epilepsy unlikely	no	1		2
	16	F	6y4m	5y11m	Some global atrophy	No epilepsy, mild intellectual impairment, history of 3 febrile convulsions	no	5		4
	17	M	2y10m	7y3m	Normal	Currently no epilepsy, history of bacterial meningitis with acute symptomatic seizures	no	22		3
	18	M	3y3m	6y0m	x	Currently no epilepsy, history of three TCS in 4 months around time of EEG	no	4		2
	19	M	13y0m	5y0m	Normal	Currently no epilepsy, but 10 years ago diagnoses of epilepsy	no	3		3
	20	M	1y8m	7y9m	Normal	Probably benign childhood epilepsy variant, exact classification unclear	no	19		0
	21	F	0y11m	7y3m	Normal	Benign infantile epilepsy syndrome	no	5		0
	22	F	4y5m	5y11m	Normal	Benign occipital epilepsy syndrome	no	41		4
IED present in EEG	23	F	7y5m	5y4m	Normal	Focal epilepsy, no etiology identified	no	5		1
	24	M	14y7m	6y5m	Normal	Focal epilepsy, no etiology identified	no	1		0
	25	F	3y10m	6y10m	Normal	Epilepsy with generalized febrile and afebrile seizures, no etiology identified	no	5		1
	26	F	8y7m	7y2m	Complete subcortical band heterotopia, mostly right temporal	Epilepsy due to bilateral subcortical band heterotopia caused by DCX mutation	no	1		0
	27	M	2y11m	5y2m	Small area of tissue damage left temporal, close to optic radiation	Epilepsy with mild developmental delay due to TANC2 mutation	valproic acid	2		1
	28	F	12y7m	7y8m	Asymmetrical Sylvian fissure with cortical dysplasia ventrocranially of left fissure	Focal epilepsy due to FCD	no	2		0
	29	F	9y10m	5y9m	Normal	Focal epilepsy, possibly atypical Rolandic (BECTS), history of frequent seizures	LVT	2		0
	30	M	4y7m	6y6m	Normal	Panayotopoulos syndrome	no	1		0
	31	M	4y1m	6y8m	Normal	Panayiotopoulos-associated seizures or vasovagal collapse	no	1	2	1
	32	M	6y2m	5y7m	Normal	Focal epilepsy, possibly atypical Rolandic (BECTS)	LVT	3	339	6
	33	M	6y7m	7y9m	Dilatation (left > right) of lateral ventricles. Small area of hyperintensity of frontal white matter	Centro-temporal (Rolandic) spikes in EEG, but no clear diagnosis of BECTS. Prematurity and developmental delay.	No		9	2 path
	34	F	11y6m	7y5m	MRI normal	Rolandic epilepsy (BECTS), just a single seizure but clear Rolandic spikes on EEG	no		3	4
	35	F	9y3m	4y11m	x	Rolandic epilepsy (BECTS), frequent seizures and 66 % IED augmentation in sleep	no		366	2
	36	M	9y11m	5y1m	Normal	Rolandic epilepsy (BECTS), frequent seizures and ESES	no		43	0
	37	F	2y3m	5y3m	Bilateral (left > right) polymicrogyria, and underdevelopment of left hemisphere	Epilepsy due to bilateral polymicrogyria caused by intrauterine CMV infection	LVT		337	4 path
	38	F	0y8m	5y8m	No hydrocephalus, but increased volume of ventral and frontal peripheral liquor areas	Epilepsy, no etiology identified, prematurity and developmental delay	no		22	0

Table 1 (continued)

#	M/F	Age at EEG	Follow-up	MRI	Diagnosis	Medication	R + VW	R + IED	R –
39	F	0y6m	7y3m	Normal	Epilepsy, no etiology identified	vigabatrin& LVT		155	25 path
40	M	0y4m	7y3m	Increased signal intensity of the cerebellum	Epilepsy, generalized seizures and episode of epileptic encephalopathy, caused by chromosomal translocation syndrome	no		125	2 path

M: male, F: female. Age at time of the sleep EEG and duration of follow-up in years (y) and months (m). MRI: at any time during follow-up. Medication at time of sleep EEG. R + VW: ripple co-occurring with vertex wave; R + IED: ripple co-occurring with interictal epileptic discharge (IED: spike or sharp wave). R -: Ripple without co-occurring event. These ripples were considered physiological unless specified with 'path' for pathological ripples. ADHD: Attention-deficit/hyperactivity disorder. TCS: tonic-clonic seizure. FCD: focal cortical dysplasia. LVT: levetiracetam. BECTS: Benign epilepsy with centro-temporal spikes. ESES: electrical status epilepticus of sleep. CMV: cyto-megalovirus. The table is sorted based on the EEG, starting with normal EEGs without IEDs and then EEGs with IEDs (first column). It is then sorted based on type of co-occurring event: vertex waves or IEDs. Finally, it is sorted based on diagnosis, starting with no diagnosis, via autism and migraine to (benign) epilepsies. Note that there were three children with (probably) benign childhood epilepsy but normal EEG (20–22), eight EEGs that contained only ripples co-occurring with vertex waves despite the presence of IEDs in the EEG (23–30), and two EEGs with ripples co-occurring with vertex waves and ripples co-occurring with IEDs (31–32) in the same EEG.

sis of ripple characteristics (duration, frequency and amplitude), but not in the analysis of ripple start versus IED start.

3.2. Ripples co-occurring with vertex waves

Children with EEGs containing ripples co-occurring with vertex waves could be divided in three subgroups: 1) children with normal EEG and no (current) diagnosis of epilepsy (1–19 in Table 1); 2) children with normal EEGs but a (probable) diagnosis of benign epilepsy (20–22); children with an EEG containing IEDs and a (probable) diagnosis of epilepsy (23–32). We first tested if the ripples in these three subgroups had the same or different characteristics. There were no statistically significant differences between the vertex wave-ripples in the three subgroups: ripple start relative to vertex wave-start: $\chi^2(2) = 3.42$, p = 0.18 (downward triangles in Fig. 2); duration: $\chi^2(2) = 2.86$, p = 0.24; frequency: $\chi^2(2) = 2.71$, p = 0.26; RMS amplitude: $\chi^2(2)$: 0.10, p = 0.95 (open circles in Fig. 3).

3.3. Characteristics of physiological or pathological ripples

Ripples started on average 103.9 ms later if the co-occurring event was a vertex wave (downward triangles in Fig. 2) than if the co-occurring event was an IED (upward triangles in Fig. 2, and Table 2). Physiological ripples (open circles in Fig. 3) had a longer duration, lower frequency, and lower amplitude than pathological ripples (black filled circles in Fig. 3, and Table 2). The differences between physiological and pathological ripples were largest for frequency and amplitude. There was no significant difference in number of oscillations, suggesting that the shorter duration of pathological ripples was due to the higher frequency of pathological ripples.

3.4. Subtypes of pathological ripples

Fig. 3 shows a bimodal distribution of the frequency of pathological ripples. A closer look at the two groups with different ripple frequencies suggested the existence of pathological ripple subtypes related to epilepsy type. 'Type 1' pathological ripples, occurring in EEGs of six children with a (possible) diagnosis of an 'idiopathic' epilepsy syndrome (Rolandic epilepsy (32–36 in Table 1) and possibly Panayiotopoulos syndrome (31)), had high frequencies (mean: 151.7 Hz), while 'Type 2' pathological ripples, occurring in EEGs of patients with symptomatic epilepsy syndromes (37–40), had lower frequencies (mean 110.5 Hz).

3.5. Differentiating physiological and pathological ripples

We classified ripples as physiological or pathological based on their RMS amplitude and frequency (Fig. 4). Of all ripples, 11.5 % were physiological (green circles in Fig. 4) and 88.5 % were pathological (type 1 (blue triangles) and type 2 (red squares) pathological ripples taken together). Mean results of the 10 repetitions of 5-fold cross validation linear discriminant analysis were: sensitivity: 0.98; specificity: 0.96; positive predictive value 0.99; negative predictive value: 0.87; accuracy: 0.98.

To further explore the potential pathological ripple subtypes, we again classified ripples using a linear discriminant analysis, but now separating physiological ripples and the two pathological ripple subgroups. Of all ripples, 11.5 % were physiological, 48.1 % were type 1 pathological ripples and 40.4 % were type 2 pathological ripples. Mean performance of the classification after 10 repetitions of 5-fold cross validation for each of the ripple types was again high: sensitivity: 0.98, 0.95, and 0.94; specificity: 0.98, 0.99, and 0.96; positive predictive value 0.86, 0.98, and 0.93; negative predictive value: 1.00, 0.95, and 0.96 for physiological ripples,
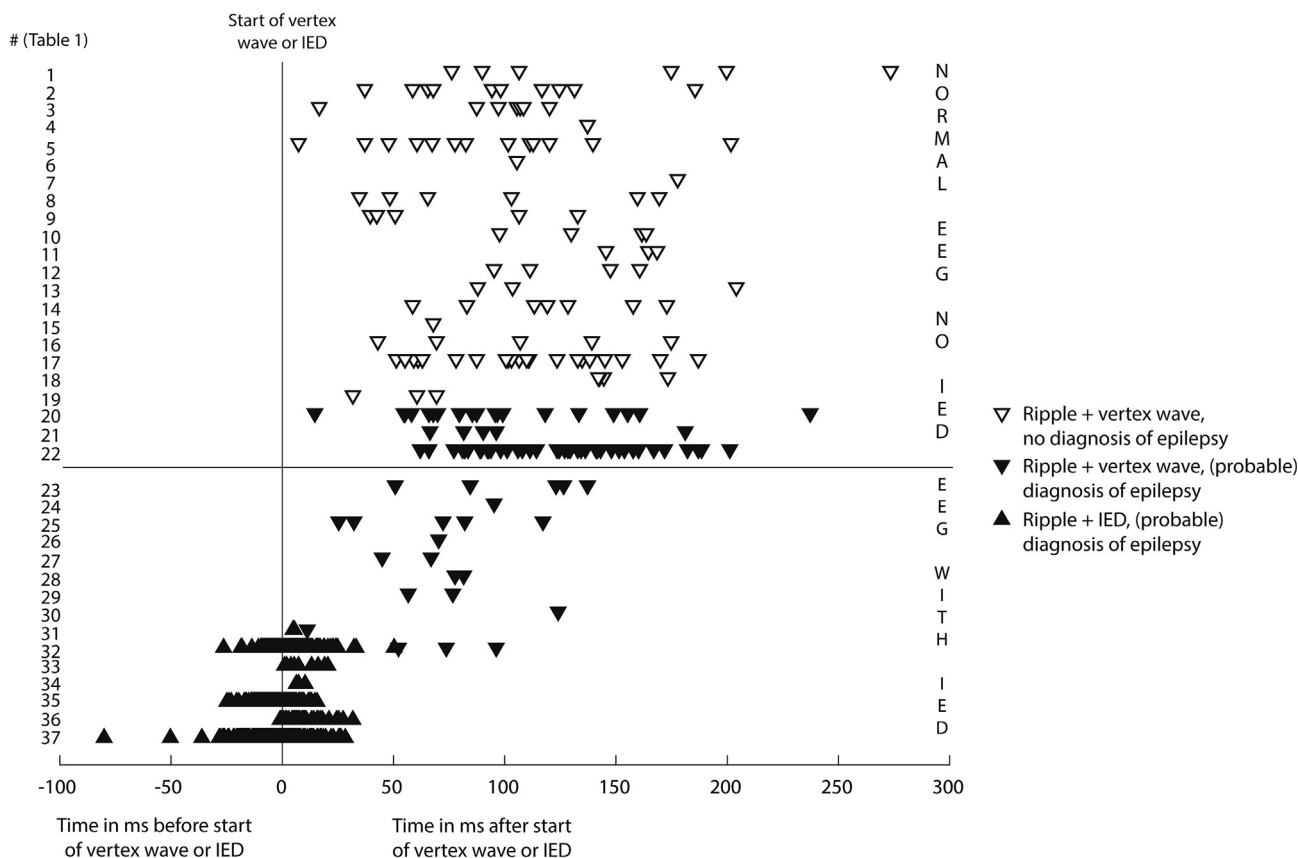
Fig. 2. Timing of ripple start relative to the start of co-occurring vertex waves or interictal epileptic discharges (IEDs: spike or sharp wave). Results are plotted following the order of Table 1. Ripples co-occurring with vertex waves are plotted as downward pointing triangles, ripples co-occurring with IEDs as upward pointing triangles. Open triangles: no diagnosis of epilepsy (only ripples co-occurring with vertex waves); black triangles: (probable) diagnosis of epilepsy. Ripples occurring in normal EEG are plotted above the horizontal line, ripples in EEGs containing IEDs are plotted below the horizontal line. Start of the vertex wave or IED is depicted as the vertical line at zero. Note that all ripples co-occurring with vertex wave occur after the start of the vertex wave, regardless of the diagnosis (open or black downward pointing triangles) or type of EEG (above or below horizontal line). Ripples co-occurring with an IED start around the start of the IED.

type 1, and type 2 pathological ripples, respectively; accuracy: 0.95.

3.6. Ripples without co-occurring events

Ripples without a simultaneously occurring sleep-specific transient or IED on the same or another channel (Table 1) mostly followed the pattern of the above mentioned ripple subgroups (Fig. 4B). Classifying these ripples using the same linear discriminant model, i.e., the model that was based on the ripples with co-occurring vertex waves or IEDs, resulted in the following performance for physiological and type 2 pathological ripples: sensitivity: 0,93 and 0.84; specificity: 0.85 and 0.93; positive predictive value 0.94 and 0.81; negative predictive value: 0.82 and 0.94; overall accuracy: 0.91. There were only two type 1 pathological ripples occurring without IED, which were both classified correctly.

4. Discussion

The results of this study suggest that physiological ripples start on average about 100 ms after the start of a co-occurring vertex wave, while pathological ripples and IEDs start approximately simultaneously. Physiological ripples marked in this study have a longer duration, lower frequency, and lower amplitude than pathological ripples. Physiological and pathological ripples could be differentiated with high accuracy based on their amplitude and frequency.

4.1. Timing of ripple start relative to the start of co-occurring IEDs or vertex waves

The observed start of ripples at approximately the same time as the start of the IED is in line with results of previous studies (Clemens et al. 2007; van Klink et al. 2016; Cai et al. 2021; Guth et al. 2021) and suggests that IEDs do not cause the generation of ripples, nor the other way around. The finding that vertex waves start before the start of the ripple suggests that they may have a facilitating role in the generation of ripples. Perhaps their role is comparable to the role of sharp waves in the sharp wave-ripple complexes that occur in the hippocampus. Hippocampal sharp waves represent a burst of activity from neurons in the CA3 region that discharges interneurons in the CA1 region. The interaction between CA1 pyramidal cells and interneurons gives rise to the ripple (Buzsáki, 2015; Buzsáki and Lopes da Silva, 2012; Gulyas and Freund, 2015). We do not know of studies that draw such a parallel between sharp waves in the hippocampus and vertex waves in the neocortex. Buszáki (2015) stated that there are similarities between the hippocampal sharp wave and the neocortical K-complex (Buzsáki 2015). K-complexes and vertex waves have the same distribution (frontal-central or central maximum near the vertex) and both can occur in response to external stimuli (Stern 2013; Halász 2016; Kane et al. 2017). The appearance of vertex waves is more similar to hippocampal sharp waves than the appearance of K-complexes, which usually have longer duration and a more complex shape (Berry et al. 2012; Stern 2013; Kane et al. 2017).
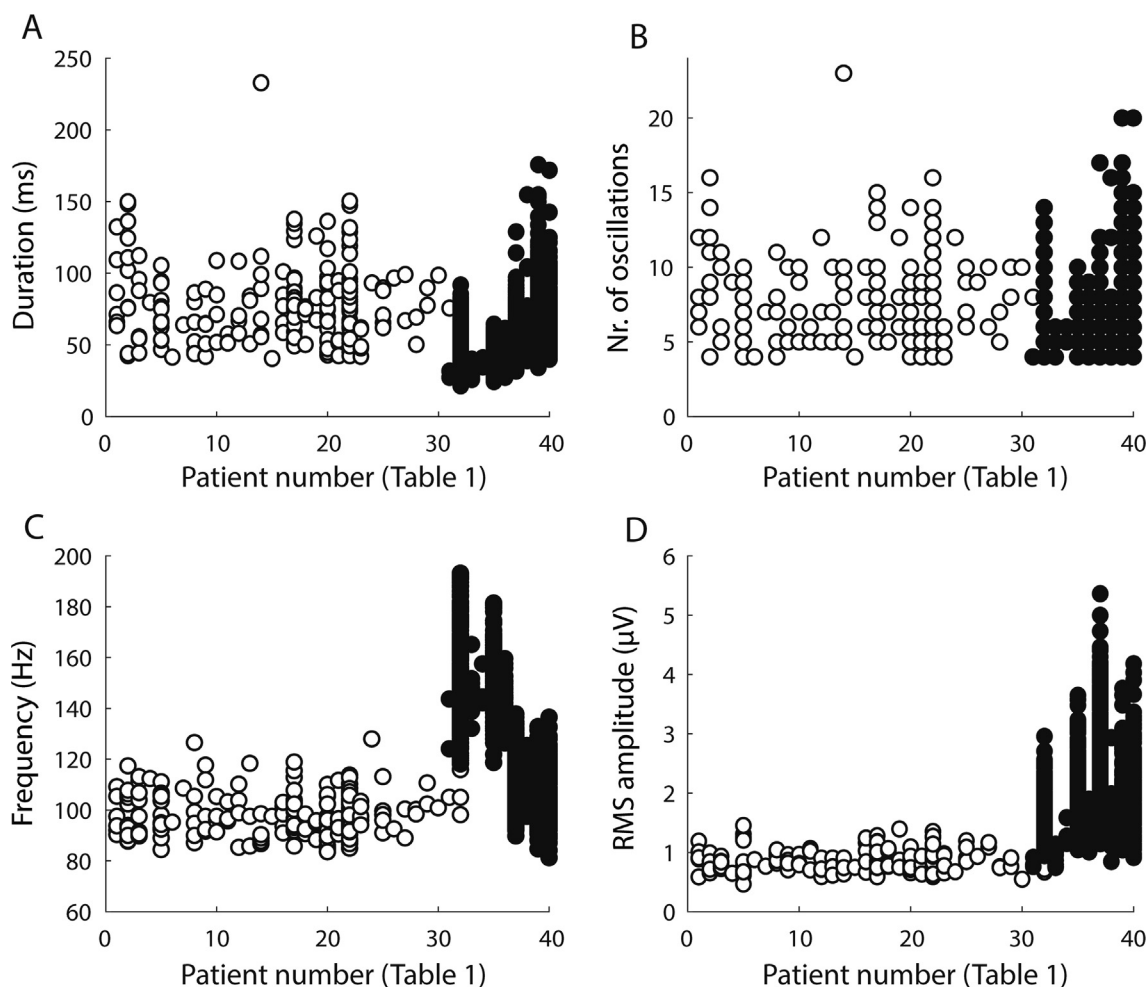
Fig. 3. Ripple characteristics plotted per child. Number on the x-axis corresponds to number in Table 1. Open circles: ripples co-occurring with vertex waves, filled black circles: ripples co-occurring with interictal epileptiform discharges (IEDs). Duration in ms. (A), number of oscillations (B), frequency in Hz (C), and root mean square (RMS) amplitude in μV (D). Note the bimodal frequency distribution for ripples co-occurring with IEDs in patient 31–36 vs 37–40 in C.

4.2. Amplitude of physiological and pathological ripples

As reported in the introduction, lower amplitude for presumed physiological ripples compared to the amplitude of pathological ripples seemed to be the most consistent result of studies on intracranially recorded ripples (Supplementary Table 1). The scalp-EEG recorded ripples in this study also showed lower amplitudes for physiological than for pathological ripples. Buzsáki and Lopes da Silva, 2012 offered an explanation for a lower amplitude of physiological ripples. They stated that, in general, the generation of physiological events is controlled by many mechanisms. As a result, the number of recruited neurons is limited, and this is reflected in the limited magnitude of physiological events. In contrast, pathological events are less well regulated. More pyramidal neurons can be involved, and recruitment can occur faster. This results in events with larger and more varying magnitude (Buzsáki and Lopes da Silva, 2012).

Between subject variation may be higher for the amplitudes of intracranially recorded ripples than for scalp-EEG recorded ripples. For example, Migliorelli et al. (2021) reported remarkable between-subject variation, which prevented them from estimating global boundaries to separate physiological and pathological ripples (Migliorelli et al. 2021). A possible explanation could be that the position of the electrodes varies between patients in iEEG. Ripples can be recorded throughout the brain, with more variation in the distance between the source of the ripples and the electrodes,

which may influence the measured amplitude. In contrast, the position of scalp electrodes is kept as similar as possible using the 10–20 system and only superficially generated ripples will be recorded. This may result in less variation in amplitude of the recorded ripples.

4.3. Frequencies of pathological ripples

A few other studies reported frequencies of scalp-EEG recorded ripples on Rolandic spikes. Similar frequencies to the ones found in this study (mean 151.7 Hz) were reported by Ikemoto et al. (2018) and Ohuchi et al. (2019), while Kobayashi et al. (2011) and Shibata et al. (2016) reported lower mean frequencies (Kobayashi et al. 2011; Shibata et al. 2016; Ikemoto et al. 2018; Ohuchi et al. 2019).

The potential existence of pathological ripple subtypes could be an explanation for the varying ripple frequencies reported in Supplementary Table 1. Methodological choices may also have affected outcomes. For example, we reported frequency based on the number of zero crossings, but we also made an estimation of the frequency from the dominant blob of a time–frequency plot. The difference between the frequencies of two pathological subgroups became larger with the time–frequency method: idiopathic epilepsy ripples had a higher frequency, while symptomatic epilepsy ripples had lower frequencies. Both methods have their strengths and weaknesses. The time–frequency based estimation was inaccurate if the wrong 'blob' was selected, for example from

Table 2
Ripple characteristics.

	Physiological ripples co-occurring with vertex waves	Pathological ripples co-occurring with IEDs	Difference and statistical significance
Ripple start relative to vertex wave or IED start (ms)	108.2 ms 95 % PI: [63.4, 153.0]	4.3 ms 95 % PI:[-40.5, 49.1]	103.9 ms 95 % CI: [82.6, 125.2] $\chi^2(1) = 38.59$, p < 0.001 Cohen's d: 5.05 Explained variance: 78.71 %
Duration (ms)	75.7 ms 95 % PI: [49.6, 101.7]	53.0 ms 95 % PI: [26.9, 79.0]	22.7 ms 95 % CI: [11.6, 33.8] $\chi^2(1) = 12.88$, p < 0.001 Cohen's d: −0.96 Explained variance: 38.75 %
Number of oscillations, transformed (ln)	1.9 oscillations *(7.0)* 95 % PI: [1.7, 2.2]	1.9 oscillations *(6.1)* 95 % PI: [1.6, 2.1]	0.1 oscillations *(0.9)* 95 % CI: [0.0, 0.2] $\chi^2(1) = 2.03$, p = 0.15 Cohen's d: −0.28 Explained variance: 8.97 %
Frequency (Hz)	98.3 Hz 95 % PI: [76.1, 120.5]	130.6 Hz 95 % PI: [108.4, 152.8]	32.3 Hz 95 % CI: [23.1, 41.6] $\chi^2(1) = 29.01$, p < 0.001 Cohen's d: 1.36 Explained variance: 62.51 %
RMS amplitude (μV)	0.9 μV 95 % PI: [0.3, 1.4]	1.8 μV 95 % PI: [1.2, 2.3]	0.9 μV 95 % CI: [0.7, 1.1] $\chi^2(1) = 30.54$, p < 0.001 Cohen's d: 1.36 Explained variance: 69.55 %

IED: interictal epileptiform discharge (spike or sharp wave). PI: predictive interval. CI: confidence interval. RMS: root mean square. The PI is the interval in which 95% of the means per participant are expected. The CI is the interval in which the true value of the parameter can be expected to occur with 95% confidence. In the above table, the PIs are based on all children in each category, while the CIs are reported for the differences between the physiological and pathological ripples. Note that all characteristics differ significantly between physiological and pathological ripples, with large effect sizes. The only exception is the number of oscillations. Analysis for number of oscillations was performed after a log transformation, because the data were skewed. The same number of oscillations for physiological and pathological ripples despite the difference of 0.1 (natural log transformed) oscillations is due to rounding to 1 decimal. Number of oscillations without log transformation is given in italics.
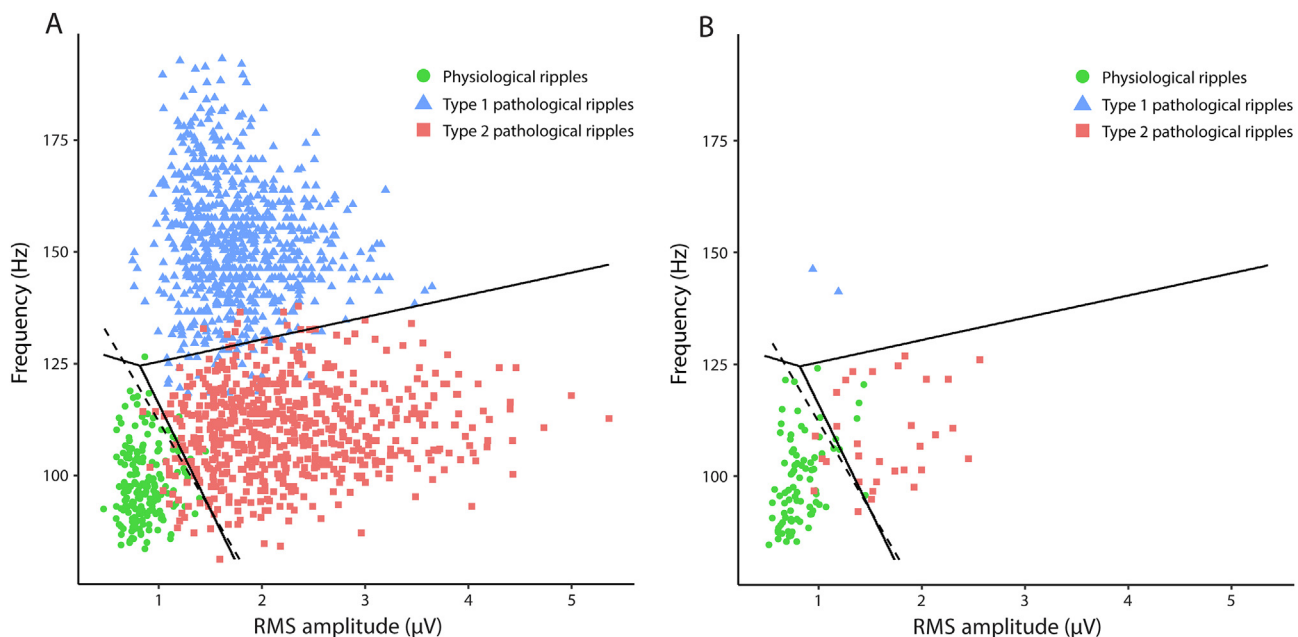

Fig. 4. Scatter plot of root mean square (RMS) amplitude in μV and frequency in Hz, showing differences between physiological ripples (green circles) and two pathological ripple subtypes (type 1, blue triangles, and type 2, red squares). Black dotted line shows the decision boundary for separating physiological and pathological ripples. Black solid line shows the decision boundary for separating physiological ripples and the two pathological ripple subtypes. The decision boundaries are based on the linear discriminant analysis of ripples co-occurring with vertex waves or interictal epileptiform discharges shown in A. The same boundaries are also shown in B, in which ripples without co-occurring event are plotted.

a noisy signal. This could result in an over- or underestimation of more than 20 Hz. The zero-crossings method also suffers from noise, which could be counted as extra zero-crossings and thus result in higher frequencies. We chose the zero-crossings method because the potential error seemed smaller than with the estimation from time-frequency plot.

4.4. Classification as physiological and pathological ripples

When both amplitude and frequency were considered, physiological and pathological ripples could be separated with high accuracy. This accuracy is remarkable when considering the results of studies on intracranial data. We mentioned some possible explanations for varying amplitudes and frequencies under 4.2 and 4.3. Another option that should be mentioned is that one can never be certain that intracranially recorded ripples that are considered physiological are truly physiological. Most studies listed in Supplementary Table 1 defined physiological ripples as occurring outside the seizure onset zone or the irritative zone (i.e. channels containing IEDs), but these ripples are still recorded in patients with chronic, drug-resistant epilepsy. In contrast, we recorded ripples in children visiting the 'First Seizure Clinic', so after a first or few episodes that might have been a seizure, but without a long standing diagnosis of epilepsy, and some of these children did not have epilepsy at all. We could therefore compare presumed physiological ripples in children with epilepsy to physiological ripples recorded in children without epilepsy. There were no differences in ripple characteristics, which makes us confident that all vertex wave-ripples reported here were indeed physiological.

The classification accuracy was mostly preserved when considering ripples without co-occurring sleep-specific transient or IED, suggesting that the differences in characteristics of physiological and pathological ripples may be a robust finding. We phrase this conclusion with caution due to the small number of ripples without co-occurring event.

4.5. Limitations

The number of ripples per EEG varied and was sometimes small, especially for ripples co-occurring with vertex waves. It is unlikely that the results of the statistical analyses would have changed if EEGs with fewer than three events could have been included, because Figs. 2 and 3 show that ripple characteristics were similar for ripples that were and ripples that were not included in the analyses.

The choice to include all ripples co-occurring with vertex waves or IEDs, thus including children regardless of their diagnosis, resulted in a heterogenous population. The timing of ripples relative to the start of IEDs was similar for different types of epilepsy, suggesting that this is a robust finding. The different frequencies of the pathological ripple subtypes were clearly visible in Figs. 3 and 4 and separable with linear discriminant analysis. We felt therefore justified in mentioning these potential subtypes, but the number of patients per subgroup was too small to draw any conclusions. Larger cohort studies are needed to investigate if pathological ripple subtypes indeed exist.

5. Conclusion

We studied physiological and pathological ripples co-occurring with two types of sharp EEG transients, vertex waves and IEDs, and observed that the timing of the start of the ripples relative to the start of vertex waves or IEDs differed. In contrast to previous iEEG studies, which found much overlap between physiological and pathological ripples, these scalp-EEG recorded ripples could be accurately separated based on their frequency and amplitude. Future studies are needed to investigate if vertex waves are neocortical equivalents of hippocampal sharp waves. Future research is also needed to investigate the existence of pathological ripple subtypes.

Declaration of interest

None.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2022.08.014.

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