Clinical Neurophysiology 132 (2021) 1452–1461

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

High-frequency oscillations recorded with surface EEG in neonates with seizures

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ARTICLE INFO

Article history:
Accepted 12 February 2021
Available online 16 April 2021

Keywords:
High-frequency oscillation
HFO
Electroencephalography
EEG
Seizure
Neonate

HIGHLIGHTS

- The neonatal brain can generate epileptic high-frequency oscillations (HFOs), though their occurrence is not common.
- Neonatal ripples and fast ripples co-occur with ictal and interictal sharp transients.
- Our data on neonatal HFOs could not yet support their potential value for outcome prediction.

ABSTRACT

Objective: Neonatal seizures are often the first symptom of perinatal brain injury. High-frequency oscillations (HFOs) are promising new biomarkers for epileptogenic tissue and can be found in intracranial and surface EEG. To date, we cannot reliably predict which neonates with seizures will develop childhood epilepsy. We questioned whether epileptic HFOs can be generated by the neonatal brain and potentially predict epilepsy.
Methods: We selected 24 surface EEGs sampled at 2048 Hz with 175 seizures from 16 neonates and visually reviewed them for HFOs. Interictal epochs were also reviewed.
Results: We found HFOs in thirteen seizures (7%) from four neonates (25%). 5025 ictal ripples (rate 10 to 1311/min; mean frequency 135 Hz; mean duration 66 ms) and 1427 fast ripples (rate 8 to 356/min; mean frequency 298 Hz; mean duration 25 ms) were marked. Two neonates (13%) showed interictal HFOs (285 ripples and 25 fast ripples). Almost all HFOs co-occurred with sharp transients. We could not find a relationship between neonatal HFOs and outcome yet.
Conclusions: Neonatal HFOs co-occur with ictal and interictal sharp transients.
Significance: The neonatal brain can generate epileptic ripples and fast ripples, particularly during seizures, though their occurrence is not common and potential clinical value not evident yet.

1. Introduction

Perinatal brain injury is often revealed by neonatal seizures (Glass and Sullivan 2009), which are accompanied by high morbidity and mortality rates (Ronen et al. 2007). Neonatal seizures are caused by a range of etiologies (Weeke et al. 2015), including hypoxic ischemic encephalopathy, genetic and metabolic disorders, and stroke. Neonatal seizures are often subclinical, especially after the administration of antiseizure medications (ASMs), and may thus go unrecognized (Boylan et al. 2002). Ictal electroencephalography (EEG) in neonates shows a range of seizure patterns, with diverse seizure morphologies and frequencies (Husain 2005). Interictal epileptiform discharges (IEDs), as seen in the adult and pediatric epileptic brain, may not be seen in neonates with sei-

Abbreviations: ASM, antiseizure medication; EEG, electroencephalography; IED, interictal epileptiform discharge; HFO, high-frequency oscillation.

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https://doi.org/10.1016/j.clinph.2021.02.400
1388-2457/

zures and cannot sufficiently distinguish between those with and without seizures (Clancy 1989). Neonatal seizures are not necessarily followed by future seizures and, to date, we cannot reliably predict which neonates with seizures will develop childhood epilepsy.

High-frequency oscillations (HFOs), consisting of ripples (80–250 Hz) and fast ripples (250–500 Hz), are promising new biomarkers for epileptogenic tissue (Frauscher et al. 2017). After their discovery in intracranial EEG, epileptic and physiological HFOs have also been found in surface EEG, mostly in children (Kobayashi et al. 2010; Mooij et al. 2017), and seem to have a diagnostic and prognostic value (van Klink et al. 2016). Recognizing HFOs in surface EEG is difficult due to artifacts caused by muscle activity and (background) noise. The co-occurrence of HFOs with IEDs helps to distinguish epileptic HFOs from physiological HFOs and artifacts.

HFOs have been associated with epileptogenesis in adult rats (Bragin et al. 2000), but neonatal HFOs have not been described yet, in either intracranial or surface EEG. High-frequency activity in the time–frequency domain - so called high-frequency (HF) augmentation (Noorlag et al. 2019) - has been reported in neonates with epileptic encephalopathy (Toda et al. 2015), but HFOs in the time domain are suggested to yield more clinically relevant information than HF augmentation (Jacobs et al. 2016; van Klink et al. 2016). We questioned whether HFOs can be generated by the neonatal brain. If so, HFOs in neonates may help us understand the process of epileptogenesis and potentially predict epilepsy.

2. Methods

2.1. Patient and EEG selection

All surface EEGs at our hospital have been recorded at a 2048 Hz sample frequency from May 2013 onwards. We selected EEGs with neonatal seizures (postmenstrual age $\leq$ 44 weeks) by evaluating EEG reports between May 2013 and May 2018. We extracted clinical data from electronic patient records. The Medical Research Ethics Committee approved the use of retrospective data for research purposes without explicit informed consent (18–354/C).

2.2. EEG recording

EEGs were recorded with the Micromed Smart Acquisition Module (Micromed,

Treviso, Italy) at a 2048 Hz sample frequency. Electrodes were placed according to the international 10–20 system adjusted for neonates (Fp2, Fp1, C4, Cz, C3, T8, T7, O2 and O1). Additional electrodes A2 and A1 were placed on the patient's ear lobes. Patients were awake, asleep or sedated and intubated.

2.3. Epoch selection

EEGs were analyzed for seizures with use of a neonatal bipolar and average montage in SystemPlus Evolution (Micromed, Treviso, Italy). We first focused on seizures to ensure a high probability of distinguishing epileptic HFOs from artifacts. Seizures were selected by one reviewer (LN) and checked by one expert in neonatal EEG (AvH) (high-pass filter 0.3 Hz; low-pass filter 70 Hz; 15 s/page; 20–100 μV/mm). A seizure was defined as an event with a pattern characterized by sudden, repetitive, evolving stereotyped waveforms with a beginning and end, which may or may not be accompanied by paroxysmal clinical changes (Pressler et al. 2018). We specified a minimal seizure duration of 10 seconds (Husain 2005).

We then annotated artifact-free interictal epochs, preferably during sleep and with IEDs, to assess the occurrence of interictal HFOs. Of note, sleep-related transients have not yet developed in the early neonatal period and may be absent in critically ill neonates (Husain 2005), and IEDs are less clear and less abundant in neonates than in adults and their (epileptic) significance is unknown (Clancy 1989; Husain 2005). We reviewed 10 minutes per patient. If multiple EEGs per patient were present, we selected the EEG with the most favorable signal-to-noise ratio and most IEDs.

2.4. HFO marking

We visually reviewed all seizures and artifact-free interictal epochs for the occurrence of HFOs with use of a bipolar montage in Stellate Harmonie (Stellate, Montreal, Canada). Automatic detection was considered undesirable because of the large numbers of artifacts. The screen was split vertically with the unfiltered signal on the left side and with an 80 Hz (finite impulse response) high-pass filtered signal on the right side for ripples (which was later switched to a 250 Hz high-pass filtered signal for fast ripples; other settings: 0.4 s/page; 1–2 μV/mm). Low-pass filters were not used. We reviewed all channels, except those with continuous artifacts or noise. EEGs in which all channels had a too poor signal-to-noise ratio were excluded. This was the case when all ictal or interictal epochs showed too much noise to reliably mark HFOs despite appropriate settings (gain for ripples: 1–2 μV/mm; gain for fast ripples: 1 μV/mm). HFOs were defined as discrete events that consist of at least four oscillations that clearly stand out from the background pattern (Noorlag et al. 2019). The unfiltered signal and - on video - head position and movements were checked to prevent marking artifacts. Candidate HFOs were marked by one reviewer (LN) and checked by two experts in HFOs (MvtK and MZ). Our policy was to include only candidate HFOs that all three reviewers consented on as being HFOs and not artifacts. If HFOs occurred on multiple channels, all were counted separately.

2.5. Data analysis

Descriptive analysis of neonates and their seizures was performed. The seizure rate was calculated with the number of seizures and the duration of EEG recordings. The number of events, their frequencies, durations and channels were derived using Matlab (R2015b (32 bits), MathWorks, Natick, MA, U.S.A). The cases with HFOs are described in detail and were compared (electrographically and clinically) among themselves and with neonates without HFOs. The relationship between the occurrence of HFOs and outcome (i.e. subsequent epilepsy and death) was assessed with Fisher's exact test (SPSS, version 25, IBM, Armonk, NY, U.S.A). Because the seizure rate was not normally distributed, its relationship with the occurrence of HFOs was assessed with the Mann-Whitney U test, and its correlation with the HFO rate with the Spearman's rank correlation coefficient.

3. Results

3.1. Patient data

In total, 138 EEGs from 104 neonates were recorded at a 2048 Hz sample frequency between May 2013 and May 2018. After excluding EEGs without seizures (n = 113) and EEGs in which all channels had a too poor signal-to-noise ratio (n = 1), 24 EEGs from 16 neonates (female: 38%; Table 1) remained. The underlying etiology was hypoxic ischemic encephalopathy in five neonates, structural (including vascular) in two, infectious in two, metabolic in two, and genetic in five. The median gestational age was 38 + 6 weeks (range 31 + 5 to 41 + 2 weeks). Two neonates were

Table 1
Clinical data. Neonate #1, 5, 7 and 15 showed ictal HFOs and #5 and 11 interictal HFOs (in bold).

Neonate #	Gender	GA (w + d)	Age @ seizures (d)	Etiology	MRI	# of seizures in all EEGs	Age @ death (d)
1.	**M**	**41 + 1**	**10**	**GBS meningitis & sepsis**	**Diffuse ischemia of the bilateral cortical gray matter and subcortical white matter, and partial thrombosis of the sagittal sinus**	**4**	**14**
2.	F	37 + 2	30	Severe intracerebral hemorrhage and diffuse ischemia due to genetic disorder (COL4A1 gene)	Diffuse ischemia, intra- and extracerebral hemorrhages and midline shift	14	34
3.	M	39 + 3	24	PIGN gene mutations	Progressive diffusion restriction in the brain stem and corticospinal tracts and global brain atrophy	2	546
4.	F	40 + 3	0	Perinatal asphyxia	Diffuse ischemia of the bilateral cortical grey matter, subcortical white matter and thalami	2	2
5.	**F**	**31 + 5**	**3**	**Presumed genetic-metabolic disorder**	**Pontocerebellar hypoplasia and bilateral intraventricular hemorrhages**	**3**	**7**
6.	M	39 + 2	0	Perinatal asphyxia	Diffuse ischemia, mostly of the bilateral subcortical white matter and basal ganglia	21	5
7.	**M**	**40 + 3**	**1**	**Perinatal asphyxia**	**(Transient) subtle cortical diffusion restriction of the bilateral Sylvian fissure and central sulcus, and small bilateral subdural and intraventricular hemorrhages**	**11**	**NA**
8.	M	38 + 2	2	GBS meningitis & sepsis	Extensive and diffuse secondary ischemia and microhemorrhages	2	8
9.	M	40 + 0	0	Perinatal asphyxia	NA	2	4
10.	M	37 + 4	45	Multiple infarctions after cardiac surgery	Multiple small areas of cerebral and cerebellar ischemia, mostly of the bilateral occipital cortex, and a few cerebellar microhemorrhages	6	NA
11.	**F**	**41 + 2**	**0**	**Molybdenum cofactor deficiency (MOSC1 gene mutations)**	**Diffuse ischemia of the bilateral cortex and basal ganglia, and hemorrhagic cystic encephalomalacia**	**6**	**7**
12.	M	38 + 2	2	GNAO1 gene mutation and hemorrhagic infarction left temporo-occipital	Diffuse ischemia, mostly of the bilateral parietal cortex, and a hemorrhagic infarction left temporo-occipital	32	246
13.	M	32 + 5	23	Tuberous sclerosis complex	A few subependymal nodules, no (sub)cortical tubers or subependymal giant cell astrocytomas*	13	NA
14.	F	40 + 3	0	Perinatal asphyxia	Severe and diffuse ischemia	38	NA
15.	**F**	**37 + 3**	**33**	**Pallister-Killian syndrome**	**NA**	**12**	**NA**
16.	M	37 + 1	1	Zellweger syndrome	Pontine hypoplasia, bilateral cerebellar heterotopias, ventriculomegaly and extensive bilateral insular polymicrogyria	7	4

d = days, F = female, GA = gestational age, GBS = group B streptococcus, HFOs = high-frequency oscillations, M = male, NA = not applicable, w = weeks.
* Due to progressive myelination, a few subcortical tubers were seen at 26 months.

born preterm (gestational age < 37 weeks). MRI of the brain was performed in 14 neonates (88%). Neonate #9 was too sick to be transported and MRI of the brain was not indicated in neonate #15, because she was already diagnosed with the Pallister-Killian syndrome. Nine neonates died within two months after birth (redirection of care because of poor prognosis in all), and two more within two years (#3 because of an out-of-hospital cardiac arrest of an unknown etiology, and #12 because of refractory epilepsy). Two (#14 and 15) of the five surviving neonates developed epilepsy and two (#7 and 13) did not. One neonate (#7) developed normally, one (#13) was mildly impaired and two (#14 and 15) were severely impaired. Neonate #10 was referred to another hospital and his clinical course is thus unknown.

3.2. EEG data

Twenty-four EEGs (range 1 to 4 per patient; total EEG duration: 19 h, 28 min and 48 s; median: 30 min and 50 s) with 175 seizures (range 1 to 27 per EEG; total seizure duration: 2 h, 44 min and 17 s) were analyzed (Table 2). The median postmenstrual age during the EEG recording was 41 + 1 weeks (range 32 + 1 to 44 + 1). The median seizure duration was 19 s (range 10 to 1188 s) and 22 out of 175 seizures (13%) were accompanied by clinical symptoms (motor: 14; non-motor: 8). Sixty-three seizures (36%) were located in the right hemisphere, 27 (15%) in the left hemisphere, 47 (27%) in both hemispheres and 38 (22%) in the midline. The median seizure rate was 7/h (range 1 to 59/h). The median percentage of EEG recordings that was occupied by seizures was 12% (range 0 to 61%). IEDs - ranging from sharp waves or spikes to aspecific interictal discharges, often of unknown (epileptic) significance - were seen in 11 of 16 neonates (69%). ASMs or sedatives were administrated shortly before or during 21 of the EEGs (88%). The background pattern was normal in seven EEGs (29%), abnormal in 13 EEGs (54%), and could not be assessed because of ongoing seizure activity in four EEGs (17%).

3.3. HFO data

Four neonates (25%) showed HFOs (5025 ictal ripples and 1427 fast ripples) during 13 seizures (7%) in six EEGs (25%) (Figs. 1–4; neonate #1, 5, 7 and 15). We found interictal HFOs (285 ripples and 25 fast ripples) in two neonates (13%) (Fig. 5; neonate #5 and 11). All HFOs were seen on channels with seizure activity and almost all co-occurred with ictal and interictal sharp transients (sharp waves or spikes). Most fast ripples were seen simultaneously with ripples.

Neonates without HFOs also showed ictal and interictal sharp transients (Fig. 5). When we compared neonates with HFOs among themselves and with those without HFOs, we could not see a relation with the seizure morphology, frequency, duration, underlying etiology, the use of ASMs or sedatives, or with whether seizures were clinical or subclinical.

The occurrence of HFOs was not related with subsequent death ($\chi^2 = 0.042$, $p = 1.00$). The seizure rate was not different in neonates

Table 2
EEG data. Neonate #1, 5, 7 and 15 showed ictal HFOs and #5 and 11 interictal HFOs (in bold).

Neonate #	Age @ EEG (d)	PMA @ EEG (w + d)	State during EEG	AMSs and sedatives before and during EEG	MV @ EEG	# of seizures	Seizure electrodes	Total seizure duration (EEG duration)	Seizure type	Ictal HFOs	Inter-ictal IEDs[1]	Inter-ictal HFOs
1.	**12**	**42 + 6**	**Unresponsive**	**PB, LDC, MDZ, LEV and MOR**	**Yes**	**1**	**C3,Fp1,C4,Fp2,T7, T8**	**17m27s (36m3s)**	**Clinical and subclinical; intermittent jerks Ri foot, both arms and lip smacking**	**Yes (R + FR)**		
1.	**13**	**43 + 0**	**Unresponsive**	**PB, LDC, MDZ, LEV, PYR and MOR**	**Yes**	**2**	**C4,T8,Fp2,Fp1,C3, Cz,T7**	**20m29s (49m17s)**	**Clinical and subclinical; intermittent jerks Ri foot**	**Yes (R + FR)**	**Yes**	**No**
1.	**13**	**43 + 0**	**Unresponsive**	**PB, LDC, MDZ, LEV, PYR, TPM and MOR**	**Yes**	**1**	**T8,C4,Fp2,T7,Fp1**	**6m29s (17m32s)**	**Clinical and subclinical; intermittent jerks Ri foot, L face and arm**	**Yes (R + FR)**		
2.	30	41 + 4	Unresponsive	PB, MDZ and MOR	Yes	2	Cz,C4	2m27s (20m20s)	Subclinical	No		
2.	32	41 + 6	Unresponsive	PB, MDZ, MOR, LDC and LEV	Yes	12	C4,Cz,T8	28m26s (46m54s)	Subclinical	No	Yes	No
3.	38	43 + 3	Moderately reactive	PB, MDZ and PYR	Yes	2	C3,T7	1m26s (34m18s)	Subclinical	No	Yes	NA[2]
4.	0	40 + 3	Irritated	None	No	2	C3,Cz	18m41s (30m57s)	Subclinical	No	No	No
5.	**3**	**32 + 1**	**Unresponsive**	**None**	**Yes**	**3**	**Fp1,Fp2**	**39 s (20m15s)**	**Subclinical**	**Yes (R + FR)**	**Yes**	**Yes (R + FR)**
6.	3	39 + 5	Unresponsive, induced hypothermia	PB, MDZ and MOR	Yes	21	C3,T7,O1,Fp1,Fp2, C4,O2, T8,Cz	6m30s (36m39s)	Subclinical	No	Yes	No
7.	5	41 + 1	Poorly reactive	PB, LDC, MDZ, MOR and PYR	Yes	10	T7,T8,O2,O1,C3,Cz	10m19s (39m24s)	Subclinical	No		
7.	**15**	**42 + 4**	**Asleep**	**PB, LDC, MDZ, MOR, PYR, LEV, PYS, FON, PHT**	**Yes**	**1**	**Cz**	**24 s (2h0m1s)**	**Clinical and subclinical; intermittent jerks L thumb**	**Yes (R)**	**No**	**No**
8.	4	38 + 6	Unresponsive	PB, LDC, MDZ and MOR	Yes	2	C4,T8,Cz,C3	3m3s (30m11s)	Subclinical	No	Yes	No
9.	1	40 + 1	Unresponsive, induced hypothermia	PB, MDZ and MOR	Yes	2	C4,O2,Cz	35 s (21m49s)	Subclinical	No	No	No
10.	46	44 + 1	Asleep, irritated	MDZ and MOR	No	6	O2,O1,T8,C4	2m14s (29m46s)	Subclinical	No	No	No[3]
11.	**4**	**41 + 6**	**Unresponsive**	**PB, LDC, MDZ, LEV and MOR**	**Yes**	**6**	**O2**	**1m31s (32m14s)**	**Subclinical**	**No**	**Yes**	**Yes (R)**
12.	8	39 + 3	Mostly awake	PB and LDC	No	6	T7,O1,O2,T8,C4,C3	5m36s (24m21s)	Clinical; moaning breathing	No		
12.	13	40 + 1	Asleep	PB, LDC, LEV, PYR and PYS	No	2	T7,O1,C3	25 s (30m42s)	Subclinical	No	Yes	No
12.	17	40 + 5	Awake and asleep	PB, LDC, LEV, PYR PYS, FOL and PHT	No	1	T7,O1,O2	13 s (31m30s)	Subclinical	No		
12.	22	41 + 3	Awake and asleep	PB, LDC, LEV, PYR PYS, FOL and PHT	No	23	T8,T7,O2,C3,C4, O1,Fp2[4]	10m11s (5h49m5s)	Subclinical (21) and clinical (2); oxygen saturation drop to 84 and 77%	No		
13.	23	36 + 0	Asleep	None	No	13	F7,T7,F8,T8,P4,C4, O1[4]	6m41s (1h0m14s)	Subclinical	No	Yes	No
14.	2	40 + 5	Unresponsive, induced hypothermia	PB, LDC, MDZ and MOR	Yes	27	Cz	6m16s (27m19s)	Subclinical	No		
14.	5	41 + 1	Unresponsive	PB, LDC, MDZ, MOR and LEV	Yes	11	Cz,C4,C3	5m4s (21m5s)	Subclinical (9) and clinical (2); jerks abdomen	No	Yes	No

(*continued on next page*)

Table 2 (*continued*)

Neonate #	Age @ EEG (d)	PMA @ EEG (w + d)	State during EEG	AMSs and sedatives before and during EEG	MV @ EEG	# of seizures	Seizure electrodes	Total seizure duration (EEG duration)	Seizure type	Ictal HFOs	Inter-ictal IEDs[1]	Inter-ictal HFOs
15.	**33**	**42 + 1**	**Lightly sedated, awake**	**MDZ and MOR**	**Yes**	**12**	**Fp2,Fp1,Fz,F4,F8, C4,T8, F3,F7,T7[4]**	**6m33s (28m49s)**	**Subclinical**	**Yes (R + FR)**	**No**	**No[3]**
16.	1	37 + 2	Unresponsive	PB and MOR	Yes	7	Diffuse electrodecrement and low amplitude paroxysmal fast activity	2m38s (30m3s)	Clinical; hiccups, stiffening L leg (tonic seizures) and frowning	No	Yes	No

ASM = antiseizure medication, d = days, FOL = folic acid, FON = folinic acid, FR = fast ripples, HFOs = high-frequency oscillations, L = left, LDC = lidocaine, LEV = levetiracetam, m = minutes, MDZ = midazolam, MOR = morphine, MV = mechanical ventilation, NA = not applicable, PB = phenobarbital, PHT = phenytoin, PMA = postmenstrual age, PYR = pyridoxine, PYS = pyridoxal sulfate, R = ripples, Ri = right, s = seconds, TPM = topiramate.

[1] Ranging from sharp waves or spikes to aspecific interictal discharges, often of unknown (epileptic) significance.

[2] Due to a signal-to-noise ratio too poor to analyze interictal HFOs.

[3] Less than 10 minutes (600 seconds) assessed due to artifacts: neonate #10: 396 seconds; neonate #15: 491 seconds.

[4] Full electrode placement according to the international 10–20 system instead of neonatal electrode placement.

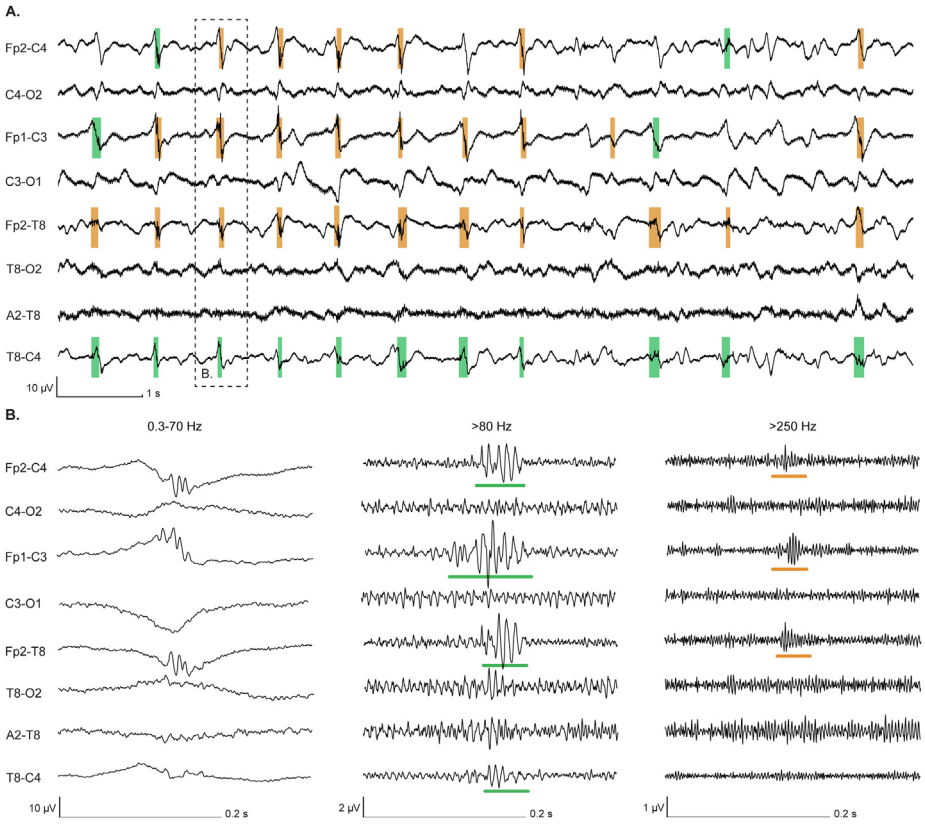

Fig. 1. EEG traces of a 12-day old boy (neonate #1), born at 41 + 1 weeks of gestation, who suffered from a group B streptococcus meningitis and sepsis. He died two weeks after birth (redirection of care after MRI diagnosis of diffuse ischemia). (A) Raw EEG (0.3–70 Hz) showing a status epilepticus. Ripples (green rectangles) and fast ripples (orange rectangles; ripples were also present at the same time points) co-occurring with ictal sharp transients were found during all four recorded seizures in his three EEGs. All channels involving Cz and T7 contained continuous artifacts and are removed in this figure. (B) Expanded raw (left) and high-pass filtered EEG showing ripples (middle; green underlined) on Fp2-C4, Fp1-C3, Fp2-T8 and T8-C4 and fast ripples (right; orange underlined) on Fp2-C4, Fp1-C3 and Fp2-T8. The signal-to-noise ratio of T8-O2 was considered too poor to mark high-frequency oscillations.

with HFOs (median 3/h; range 1 to 25/h) compared to those without HFOs (median 12/h; range 2 to 59/h) (U 32.00, p = 0.09), and the HFO rate in neonates with HFOs was not significantly correlated with the seizure rate (ictal ripple rate: ρ = -0.35, p = 0.09; ictal fast ripple rate: ρ = -0.23, p = 0.28; interictal ripple rate: ρ = 0.04, p = 0.84; interictal fast ripple rate: ρ = 0.02, p = 0.94). The relationship between the occurrence of HFOs and subsequent epilepsy could not be reliably assessed due to the small number of survivors.

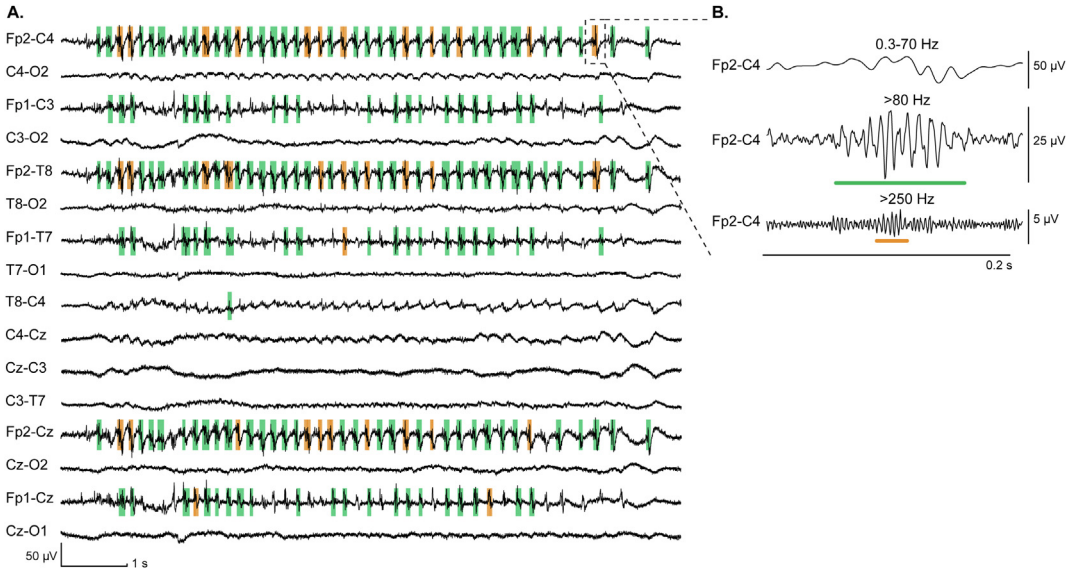

Fig. 2. EEG traces of a 3-day old girl (neonate #5), born at 31 + 5 weeks of gestation, with a prematurity-related bilateral intraventricular hemorrhage on ultrasound and burst suppression on 2-channel amplitude-integrated EEG. She died one week after birth (redirection of care after MRI diagnosis of pontocerebellar hypoplasia, presumably due to a genetic-metabolic disorder). (A) Raw EEG (0.3–70 Hz) showing a frontopolar seizure. Ripples (green rectangles) and fast ripples (orange rectangles; ripples were also present at the same time points) co-occurring with ictal sharp transients were found during all three seizures. A2-T8 and T7-A1 (both without seizure activity) are removed in this figure. (B) Expanded raw (top) and high-pass filtered EEG showing a ripple (middle; green underlined) and fast ripple (bottom; orange underlined) on Fp2-C4.

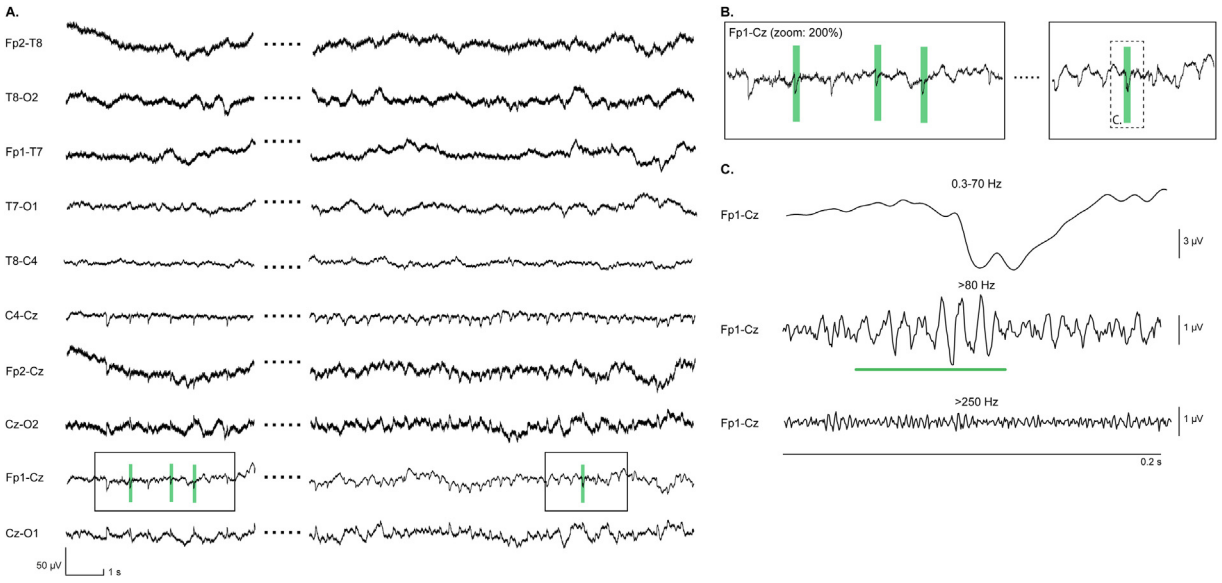

Fig. 3. EEG traces of a 15-day old boy (neonate #7), born at 40 + 3 weeks of gestation, who suffered from perinatal asphyxia. He is currently six years old and showed a remarkable recovery; he developed normally after an initial mild developmental delay and remained seizure-free after anti-seizure medications were stopped at the age of 20 months. (A and B) Raw EEG (0.3–70 Hz) showing a central seizure. Ripples (green rectangles) co-occurring with ictal sharp transients were found on Fp1-Cz. No fast ripples were found. All channels involving C3 contained continuous artifacts and are removed in this figure. In addition, Fp2-C4, C4-O2, A2-T8 and T7-A1 (all without seizure activity) have been removed. (C) Expanded raw (top) and high-pass filtered EEG showing a ripple (middle; green underlined) on Fp1-Cz, but no fast ripple (bottom).

3.3.1. HFO findings in individual cases

Neonate #1 showed HFOs during all four recorded seizures in his three EEGs (total seizure duration: 44 min and 25 s; total EEG duration: 1 h, 42 min and 52 s). We found 4080 ictal ripples (mean rate 92/min; mean frequency 135 Hz; mean duration 62 ms) and 1212 fast ripples (mean rate 27/min; mean frequency 295 Hz; mean duration 24 ms). Rates were 57, 137 and 44/min for ripples in the first, second and third EEG, respectively, and 8, 47 and 17/min for fast ripples. The channels with HFOs were all channels involving Fp2, Fp1, T8 and T7 (Fig. 1). Neonate #1 was a boy, born at 41 + 1 weeks of gestation, and suffered from a group B streptococcus meningitis and sepsis. He had a status epilepticus in all his three EEGs, of which the first was recorded 12 days after birth, and the second and third EEG the day after. He died two weeks after birth.

Neonate #5 showed HFOs during all three seizures in her only EEG (total seizure duration: 39 s; EEG duration: 20 min and 15 s). We found 750 ictal ripples (mean rate 1154/min; mean frequency 132 Hz; mean duration 93 ms) and 142 fast ripples (mean rate 218/min; mean frequency 310 Hz; mean duration 30 ms). The

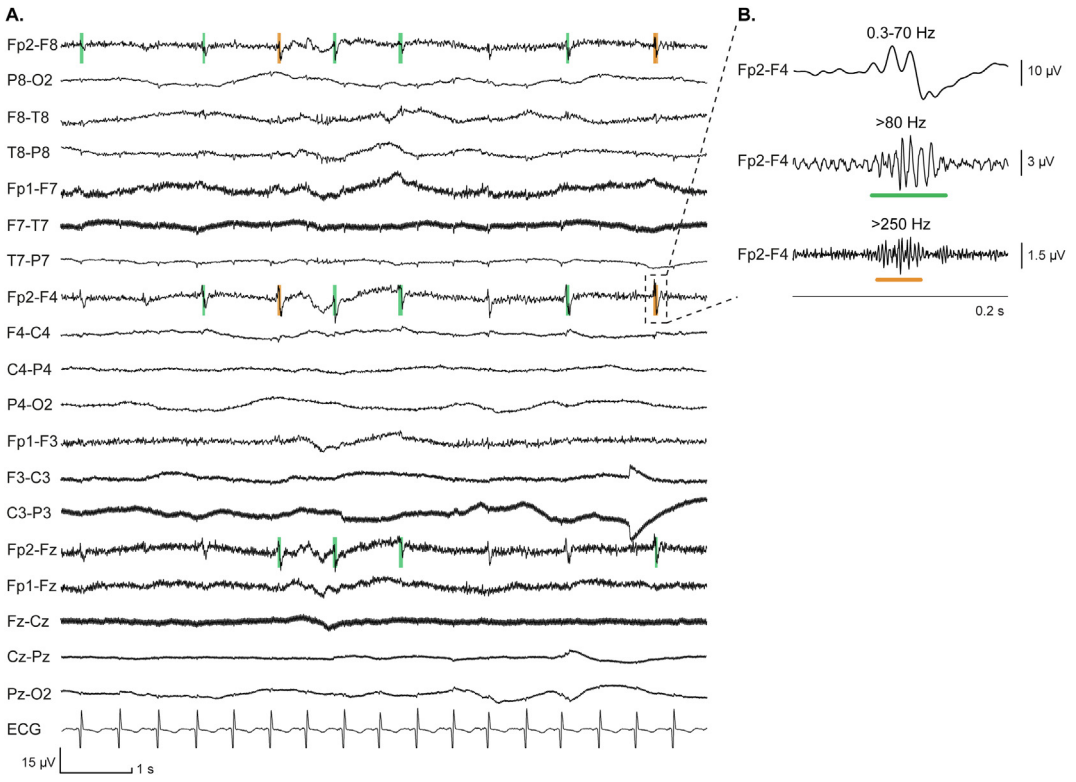

Fig. 4. EEG traces of a 33-day old girl (neonate #15), born at 37 + 3 weeks of gestation, who was diagnosed with Pallister-Killian syndrome. She developed epilepsy and had a severe developmental delay. (A) Raw EEG (0.3–70 Hz) showing a seizure consisting of frontopolar spikes. Ripples (green rectangles) and fast ripples (orange rectangles; ripples were also present at the same time points) co-occurring with ictal spikes were found on all channels involving Fp2. ECG is also shown, to illustrate that the time points of frontopolar spikes and QRS complexes are largely different. All channels involving O1 contained continuous artifacts and are removed in this figure. (B) Expanded raw (top) and high-pass filtered EEG showing a ripple (middle; green underlined) and fast ripple (bottom; orange underlined) on Fp2-F4.

channels with HFOs were all channels involving Fp2 and Fp1, and a few times T8-C4 (Fig. 2). In 10 minutes, we found 228 interictal ripples (mean rate 23/min; mean frequency 124 Hz; mean duration 67 ms) and 25 fast ripples (mean rate 3/min; mean frequency 300 Hz; mean duration 18 ms) on the same channels during bursts, sharp waves and spikes (Fig. 5), but not during suppressions. Not all bursts, sharp waves and spikes contained HFOs. We noticed that HFOs during sharp transients (sharp waves or spikes) were more pronounced than those during bursts. Neonate #5 was a girl, born at 31 + 5 weeks of gestation, and the EEG was recorded three days after birth. She presumably had a genetic-metabolic disorder and showed pontocerebellar hypoplasia and a prematurity-related bilateral intraventricular hemorrhage on MRI. She died one week after birth.

Neonate #7 showed HFOs during one seizure (seizure duration: 24 s; EEG duration: 2 h, 0 min and 1 s) in one of his two EEGs. We found four ictal ripples (rate 10/min; mean frequency 136 Hz; mean duration 65 ms) and no fast ripples. The channel with ripples was Fp1-Cz (Fig. 3). Neonate #7 was a boy, born at 40 + 3 of gestation, and the EEG with one seizure with HFOs was recorded 15 days after birth, which was 10 days after the EEG with 10 seizures without HFOs (seizure duration: 10 min and 19 s; EEG duration: 39 min and 24 s). The seizures with and without HFOs consisted of sharp waves, but the one seizure with HFOs - when compared to the 10 seizures without HFOs - was on different channels (respectively, channels involving Cz vs. channels involving T8 and T7), slightly faster (1,5 to 4 Hz vs. 1 to 2 Hz) and partially with clinical symptoms (intermittent jerks of the left thumb). He suffered from perinatal asphyxia and induced hypothermia was applied twice because of ongoing seizure activity. He survived and was seizure-free after the neonatal period. He developed normally after an initial mild developmental delay and ASMs were stopped at the age of 20 months.

Neonate #15 showed HFOs during five out of 12 seizures in her only EEG (EEG duration: 28 min and 49 s). Seven seizures consisted of frontocentrotemporal delta activity and contained no HFOs (seizure duration: 3 min and 54 s), and five seizures consisted of frontopolar spikes and contained ripples and fast ripples (seizure duration: 2 min and 39 s). We found 191 ictal ripples (mean rate 72/min; mean frequency 129 Hz; mean duration 57 ms) and 73 fast ripples (mean rate 28/min; mean frequency 322 Hz; mean duration 25 ms). The channels with HFOs were all channels involving Fp2 and Fp1, and few times F8-T8 (Fig. 4). Neonate #15 was a girl, born at 37 + 3 weeks of gestation. She was diagnosed with Pallister-Killian syndrome, developed epilepsy and had a severe developmental delay. Later on, she was referred to another hospital and her further clinical course is thus unknown.

Neonate #11 showed interictal ripples, but no ictal HFOs. In 10 minutes, we found 57 ripples (rate 6/min; mean frequency 172 Hz; mean duration 50 ms) and no fast ripples. Neonate #11 was a girl, born at 41 + 2 weeks of gestation. The EEG was recorded four days after birth and contained six right occipital seizures (total seizure duration: 1 min and 31 s) and no ictal HFOs. Interictally, the EEG showed bursts, sharp waves, spikes and aspecific interictal discharges. HFOs were consistently seen on left centrotemporal sharp waves (C3 and T7) (Fig. 5). Neonate #11 was diagnosed with molybdenum cofactor deficiency due to MOSC1 gene mutations, and MRI showed diffuse ischemia of the bilateral cortex and basal ganglia, and hemorrhagic cystic encephalomalacia. She died one week after birth.

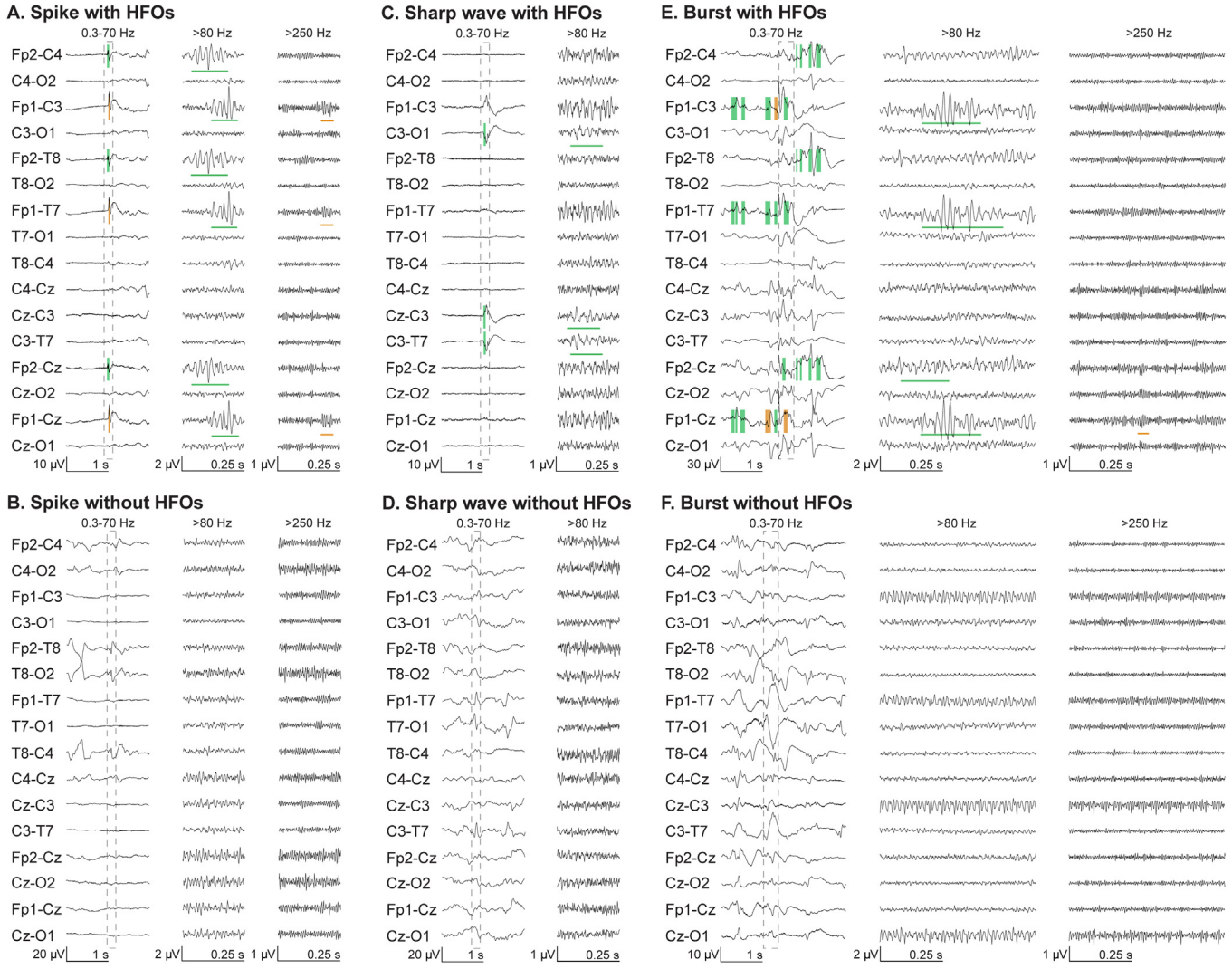

Fig. 5. Examples of interictal transients with and without high-frequency oscillations (HFOs). (A) Spike with ripples and fast ripples (neonate #5). Raw EEG (0.3–70 Hz), ripple (>80 Hz) and fast ripple (>250 Hz) setting are shown. (B) Spike without HFOs (neonate #1). (C) Sharp wave with ripples (neonate #11), but no fast ripples. Raw EEG (0.3–70 Hz) and ripple (>80 Hz) setting are shown. All channels involving Fp1 contain continuous noise. (D) Sharp wave without HFOs (neonate #12). (E) Burst with ripples and one fast ripple (neonate #5). Raw EEG (0.3–70 Hz), ripple (>80 Hz) and fast ripple (>250 Hz) setting are shown. (F) Burst without HFOs (neonate #8). Fp1-C3, Cz-C3, Cz-O1 and to a lesser extent Fp1-T7 contain continuous noise, a frequently encountered problem in neonatal surface EEG.

4. Discussion

This study reports, for the first time, the detection of epileptic HFOs in surface EEG of neonates. We described four cases (25%) with neonatal HFOs during thirteen seizures (7%) out of 16 neonates with 175 seizures. Interictal HFOs were also found in two cases (13%). We showed that epileptic HFOs can be generated by the neonatal brain, particularly during seizures, though their occurrence is not common.

Our numbers are too small to draw conclusions on the pathophysiological meaning or clinical value of neonatal HFOs, particularly because only five of the 16 neonates survived. Three out of five neonates with HFOs died within two months after birth, as did six out of 11 without HFOs. In addition, the cases with HFOs were heterogeneous with respect to EEG findings, postmenstrual age and underlying etiology. Interestingly, we found HFOs in one seizure of neonate #7, who had an earlier EEG with 10 seizures without HFOs, and in five out of 12 seizures of neonate #15, who had two seizure patterns (frontopolar spikes with HFOs, and frontocentrotemporal delta activity without HFOs).

Neonatal seizure patterns are different from the typical ones that are seen in children and adults (Perucca et al. 2014), and characteristics may even vary between two seizures within one neonate, ranging from simple sinusoidal waves to complex polyspike seizure patterns and from delta to bèta (Husain 2005). The etiology of neonatal seizures also differs from that in children and adults (Pressler et al. 2018), and neonatal seizures are more often caused by lesions affecting large parts of the brain, such as hypoxic ischemic encephalopathy (Weeke et al. 2015). A recent study reported that the median seizure duration in term neonates is 109 s (interquartile range 65.0 to 225.5 s) (Rennie et al. 2019), and seizures are often shorter in preterm neonates (Janáčková et al. 2016). We found a median seizure duration of 19 s, which is notably shorter. This may be due to the administration of ASMs shortly before or during most EEGs (88%) (van Rooij et al. 2010). This may in turn be because, in our hospital, neonates at risk are continuously monitored with 2-channel amplitude-integrated EEG. Multichannel EEG is recorded when they are suspected of having seizures (either clinically or electrographically), but often preceded by the administration of ASMs.

Neonatal seizures are often subclinical after ASMs have been administered (Boylan et al. 2002); a process also called "uncoupling". ASMs and sedatives affect the seizure rate and duration (van Rooij et al. 2010), but also the background pattern and presumably the HFO rate. Previous studies have shown that the HFO rate decreases after the administration of propofol (Zijlmans et al. 2012), diazepam (Toda et al. 2013), various other ASMs (Zijlmans et al. 2009), and also after treatment with adrenocorticotropic hormone (Kobayashi et al. 2015) and methylprednisolone (Gong et al. 2018). The effect of other sedatives and the ASMs mostly used in neonates - phenobarbital, lidocaine, midazolam, levetiracetam and pyridoxine - on HFOs has not been studied yet, but we know that they may suppress the background pattern (Shany et al. 2008; Bok et al. 2010; Deshpande et al. 2021), especially in critically ill neonates, so one could expect a similar effect on the HFO rate.

Epileptic HFOs are thought to result from in-phase firing of groups of neurons in case of ripples and desynchronized firing in case of fast ripples (Foffani et al. 2007). They have mostly been studied in intracranial EEG of the mesial temporal areas, but can be found in any part of the cortex with an underlying pathology (Jacobs et al. 2009). Physiological HFOs in intracranial EEG recordings mostly occur in the eloquent areas, such as the pre- and postcentral gyrus and the occipital cortex (Frauscher et al. 2018). Recently, physiological HFOs have been discovered in pediatric surface EEG during sleep and were predominantly found in the central areas (Mooij et al. 2017). One could hypothesize that the neonatal brain may be relatively incapable of generating HFOs, neither epileptic nor physiological, because the neonatal brain has not matured and myelinated yet. In addition, the background pattern of neonates shows less fast activity than the adult brain (Vanhatalo and Kaila 2006). Conversely, one could expect a high probability of finding HFOs in neonatal surface EEG, because of their thin and unossified skulls and incomplete cortical folding, especially in preterm neonates.

We expected the highest probability of finding epileptic HFOs during sharp seizure patterns, because (1) ictal epochs show more HFOs than interictal epochs in intracranial EEG recordings (Zijlmans et al. 2011), (2) seizure patterns with a high frequency of sharp transients in intracranial EEG correlate with a high HFO rate (Ferrari-Marinho et al. 2016), and (3) up to 63% of interictal HFOs co-occur with spikes in pediatric and adult surface EEG (Andrade-Valenca et al. 2011; Melani et al. 2013; Qian et al. 2016). In one of the four neonates with ictal HFOs (#5), we also found interictal ripples and fast ripples during sharp transients and bursts. This convinced us even more of the authenticity of the ictal HFOs. The discovery of interictal HFOs during bursts seems in agreement with a previous study of Toda et al. (Toda et al. 2015), wherein HF augmentation was reported during bursts in neonates with epileptic encephalopathy. A few bursts in neonate #5 were also accompanied by high-frequency activity, which did not meet the definition of HFOs.

Fast ripples may be more specific for epileptogenic tissue than ripples in intracranial EEG (van 't Klooster et al., 2015). Fast ripples in scalp EEG of children with tuberous sclerosis complex correlated with cortical tubers as seen on MRI (Bernardo et al. 2018). We did not find this in our study population; neonate #1 (bilateral frontopolar fast ripples) showed diffuse brain injury, #5 (mostly right frontopolar fast ripples) showed no corresponding lesion, and MRI of the brain was not performed in neonate #15 (bilateral frontopolar fast ripples). Cuello-Oderiz et al. studied the influence of the location of a lesion on the occurrence of IEDs and HFOs in adults with focal epilepsy. Ripples were almost exclusively found in patients with superficial lesions (Cuello-Oderiz et al. 2017). In our study population, this could also be suspected in one neonate (#7) with only ripples on Fp1-Cz; he showed transient subtle cortical diffusion restriction - suggestive of transient focal ischemia - of the bilateral central sulcus. Neonate #11 (left centrotemporal ripples), however, showed diffuse brain injury on MRI.

Previous studies have shown a positive correlation between HFO rate and seizure frequency (van Klink et al. 2016; Boran et al. 2019; Kramer et al. 2019). Seizure frequency is more difficult to assess in neonates, because clinical symptoms are often misinterpreted as neonatal seizures (Murray et al. 2008). Furthermore, neonatal seizures are often subclinical and may thus go unrecognized, even when neonates are continuously monitored with 2-channel amplitude-integrated EEG, since short and low-amplitude seizures can be missed (Rakshasbhuvankar et al. 2020). Therefore, we used seizure rate on multichannel EEG as a replacement for seizure frequency to assess a correlation with HFO rate. Seizure rate and HFO rate were not correlated. Of note, we used relatively short EEG recordings (EEG duration ranged between 17 min and 32 s and 5 h, 49 min and 5 s, with a median of 30 min and 50 s), and seizure rate would be more reliably estimated with prolonged EEG.

An important limitation of our study is the subjectivity of the visual review of HFOs in surface EEG recordings with large numbers of artifacts. HFOs are more difficult to recognize in surface EEG than in intracranial EEG. The chance to find them is highest when the signal-to-noise ratio is favorable (von Ellenrieder et al. 2014) and the cortical source superficial (Cuello-Oderiz et al. 2017). The signal-to-noise ratio may theoretically be better in neonates due to their thin and unossified skulls, but also suffers from artifacts caused by muscle activity and external sources (André et al. 2010). The latter factor may have contributed significantly to the large numbers of artifacts in our study population, since many neonates received external support, such as mechanical ventilation, drips and heat lamps. Three authors reviewed candidate HFOs to avoid false-positive and -negative detections, and we particularly focused on HFOs that co-occurred with ictal and interictal sharp transients, because up to 63% of interictal HFOs co-occur with spikes (Andrade-Valenca et al. 2011; Melani et al. 2013; Gong et al. 2018). Contradictory to what one may expect, we did not see more muscle artifacts in ictal epochs than in interictal epochs, probably because neonatal seizures were often subclinical (87% in our manuscript). In previous studies in older patients, we circumvented artifacts from muscle activity by selecting epochs during sleep, but sleep-related transients have not yet developed in the early neonatal period and may be absent in critically ill neonates (Husain 2005). In addition, most EEGs (88%) may have been affected by the previous or concurrent administration of ASMs and sedatives, complicating the classification of sleep.

In future studies, it would be interesting to try to improve the signal-to-noise ratio (for example with subdermal electrodes (Pizzo et al. 2016)) and spatial resolution, to potentially find HFOs in more neonates, and to record multichannel EEG earlier to study the effect of ASMs and sedatives on neonatal HFOs. Ideally, we would want to study the role of neonatal HFOs in epileptogenesis and outcome, for example the development of epilepsy, but this may be too difficult in neonatal EEG due to the poor signal-to-noise ratio. In healthy neonates, with presumably less external sources artifacts and in whom epochs with sleep can be selected, it would be interesting to study whether the neonatal brain can also generate physiological ripples and if not, from which age onwards the brain can generate them.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

LN is supported by the Wilhelmina Children's Hospital research fund 2016-2017 (D-17-010011).

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