Variation of scalp EEG high frequency oscillation rate with sleep stage and time spent in sleep in patients with pediatric epilepsy

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Objective: High frequency oscillations (HFO) in scalp EEG are a new and promising epilepsy biomarker. However, considerable fluctuations of HFO rates have been observed through sleep stages and cycles. Here, we aimed to identify the optimal timing within sleep and the minimal data length for sensitive and reproducible HFO detection.

Methods: We selected 16 whole-night scalp EEG recordings of paediatric patients with a focal structural epilepsy. We used an automated clinically validated HFO detector to determine HFO rates (80–250 Hz). We evaluated the reproducibility of HFO detection across intervals.

Results: HFO rates were higher in N3 than in N2 and REM sleep and highest in the first sleep cycle, decreasing with time in sleep. In N3 sleep, the median reliability of HFO detection improved significantly from 67% (interquartile range: iqr 57) to 78% (iqr 59) to 100% (iqr 70%) for 5-, 10-, and 15-min data intervals, improving significantly (p = 0.004, z = 2.9) from 5 to 10 min but not from 10 to 15 min.

Conclusions: We identified the first N3 sleep stage as the most sensitive time window for HFO rate detection. At least 10 min N3 data intervals are required and sufficient for reliable measurements of HFO rates.

Significance: Our study provides a robust and reliable framework for scalp HFO detection that may facilitate their implementation as an EEG biomarker in paediatric epilepsy.

1. Introduction

High frequency oscillations (HFO) in scalp EEG are a new and promising non-invasive epilepsy biomarker providing added prognostic value, particularly in the paediatric population (Boran et al., 2019; Cserpan et al., 2021; Nariai et al., 2020; Ohuchi et al., 2019; Tsuchiya et al., 2020). Beyond the initial use of HFO to delineate the epileptogenic zone in epilepsy surgery, HFO are currently investigated as potential biomarkers of epileptogenesis, seizure propensity, disease severity, and treatment response (Gotman, 2021; Jacobs and Zijlmans, 2020). The utility of scalp HFO as EEG-
biomarker in paediatric epilepsy has been substantiated by recent studies corroborating the correlation of scalp HFO rates with (1) epileptogenesis after a first epileptic seizure, regardless of aetiology (Klootz et al., 2021), (2) seizure propensity in the presence of a predisposing condition such as centrotemporal spikes (Kramer et al., 2019) or tuberous sclerosis (Bernardo et al., 2018), (3) disease severity in focal structural epilepsy (Boran et al., 2019) as well as in a wide range of paediatric epilepsy syndromes (Iremoto et al., 2018; van Klink et al., 2016; Narai et al., 2020; Toda et al., 2015), and (4) treatment response following the administration of anti-seizure medication or epilepsy surgery (Boran et al., 2019; Kobayashi et al., 2015). HFO analysis in patients with epilepsy is typically restricted to random and relatively brief time periods of mostly 5–30 min, even in those undergoing long-term EEG recordings (Zelmann et al., 2014). However, the question remains whether all available data, over several nights, should be utilised for analysis or whether carefully selected intervals suffice for clinically meaningful results (Fedele et al., 2019). Data selection will have to balance the need for stable estimates of, e.g., HFO localisation patterns and rates that accurately reflect network properties (Chen et al., 2021; Fedele et al., 2019; Gliske et al., 2018) against the benefits of shorter intervals making this approach more widely applicable, even in short standard EEGs. This makes the appropriate choice of the most suitable time windows and sample size to ensure data quality and representativity essential.

HFO analysis is routinely performed in sleep to avoid contamination by muscle artefacts (Zijlmans et al., 2017). However, considerable fluctuations of HFO rates have been observed across sleep stages and cycles (Bagshaw et al., 2009; Dümppelmann et al., 2015; von Ellenrieder et al., 2017; Gliske et al., 2018; Staba et al., 2004), analogous to the significant modifications in the rates of spikes, the standard EEG-biomarker of epilepsy. For spikes, a meta-analysis based on both scalp and invasive EEG revealed higher occurrence rates in NREM (N3) sleep compared to other vigilance states (Ng and Pavlova, 2013). Similarly, pathological HFO rates were shown to be highest during NREM sleep in invasive EEG studies in drug-resistant focal epilepsy (Al-Bakri et al., 2018; Bagshaw et al., 2009; von Ellenrieder et al., 2017; Staba et al., 2004) and, most importantly, pathological HFO during NREM sleep were shown to best localise the epileptogenic zone (Klimes et al., 2019). Furthermore, pathological HFO rates have recently been shown to decrease with accumulated time in sleep in invasive recordings (von Ellenrieder et al., 2017), pointing to data from the first sleep cycle as being most suitable for analysis, allowing for the most sensitive detection of HFO. However, it is unclear if findings from invasive EEG in mainly adult cohorts with drug-resistant focal epilepsy undergoing presurgical evaluation apply to scalp EEG recordings across childhood and adolescence and in a broader range of epilepsy syndromes. The relationship between sleep and scalp HFO characteristics in paediatric epilepsy remains insufficiently explored, despite being crucial for implementing HFO as a clinical tool. Yet, it is imperative to characterize the impact of different parameters on HFO rate variability, including those fluctuating over weeks or months, such as patient age and epilepsy severity, and those fluctuating over hours and minutes, such as sleep cycles and stages. The interpretation of HFO rates requires a valid framework integrating multiple key parameters and thus facilitating reliable comparisons between different patients and at different time points.

In the present study, we aimed to identify the optimal timing within sleep and the minimal data length for sensitive and reproducible HFO detection. To address the hypotheses that scalp HFO rate is highest in the N3 sleep stage and decreases with accumulated time in sleep, we retrospectively analysed whole-night scalp EEG from paediatric focal structural epilepsy patients, implementing a previously validated automated HFO detector (Boran et al., 2019; Fedele et al., 2017, 2016; Cserpan et al., 2021, 2016). To address the hypothesis that at least 10 min of N3 sleep should be analyzed to ensure a reliable scalp HFO detection in pediatric epilepsy, we applied the test-retest reliability methodology (Fedele et al., 2017).

2 Methods

2.1 Patient recruitment

We recorded whole-night video-EEGs from 72 children and adolescents (<18 y) with epilepsy at the University Children’s Hospital Zurich between January 2020 and January 2021. For the current study focusing on the effect of sleep homeostasis on scalp HFO rates, we included patients with 1) the diagnosis of focal epilepsy of structural aetiology, based on electroclinical correlations and imaging findings, 2) availability of a whole-night scalp EEG recording obtained at a high sampling frequency (>1000 Hz). We excluded patients 1) with seizures during the recording night, and 2) with excessive technical or biological artefacts throughout the recording night. The clinical indication for whole-night EEG in these patients included presurgical evaluation and treatment monitoring.

The collection of patient data and the scientific analysis were approved by and performed according to the guidelines and regulations of the local ethics committee (Kantonale Ethikkommission Zürich, KEK-ZH PB-2021–00770). All patients and their parents gave written informed consent before participating in the study.

2.2. Scalp EEG recording & data selection

Patients underwent whole-night video-EEG with 21 electrodes placed according to the international 10–20 system at a 1024 Hz sampling rate using the Micromed® EEG recording system (Mogliano Veneto, Treviso, Italy). Impedances were typically < 5 kΩ. Sleep stages, including NREM stages N1, N2, N3 and REM (rapid eye movement) sleep were marked by experienced neurophysiologists according to the American Academy of Sleep Medicine (AASM) (Berry et al., 2017), taking into account available electrooculography and electromyography channels in addition to EEG, as recommended by the AASM. We selected only N2, N3, and REM sleep stages for further analysis since muscle activity and movement artefacts in wakefulness interfere with HFO detection, leading to increased false positives.

We divided the selected data into 5-min intervals for further processing. During visual pre-processing, 5-min EEG intervals heavily contaminated by artefacts and channels with continuous interference were fully excluded from further analysis. Further artefacts were detected and excluded during the automated HFO detection, as detailed below.

HFO detection and analysis were performed blinded to the clinical characteristics of the patients, and the results from HFO analysis were not considered for clinical decision making.

2.3 Automated HFO detection

To capture the HFO activity with the highest possible spatial resolution, we re-referenced to a bipolar montage using all combinations of neighbouring electrodes and thus obtained 52 bipolar channels.

Scalp HFO detection was conducted with a clinically validated, automated HFO detector (Boran et al., 2019; Burnos et al., 2014; Cserpan et al., 2021; Fedele et al., 2017, 2016) that operates in three stages, as previously described in detail (Fedele et al., 2016). The detector uses Stockwell’s algorithm (Stockwell et al., 1996) for the time–frequency transform of the EEG signal (Burnos et al., 2014). In Stage I, baseline detection, we determine a baseline amplitude threshold in artefact-free time intervals by
selecting epochs of high Stockwell entropy (low frequency oscillatory activity) in the ripple band (80–250 Hz). Events exceeding this threshold are marked as events of interest (EoI). In Stage II, HFO validation by Stockwell transform, the detector selects those EoI where a high-frequency peak is isolated from the low-frequency activity and marks them as HFO. This step reflects the assumption that HFO are brief events with a distinct high frequency contribution that stands out from the baseline (Burnos et al., 2014). In Stage III, artefact rejection, the detector screens EoI that have been selected in stage II to further eliminate artefacts. This step is guided by the assumption that HFO are generated by highly localized cortical tissue and should thus not be detectable across hemispheres (von Ellenrieder et al., 2014). Thus, the detector rejects all EoI co-occurring in homologous channels of the two hemispheres, in addition to those with amplitude ≥40 μV or signal-to-noise ratio < 4. No further visual validation of the events is performed, rendering the algorithm fully automated.

The HFO detector was thus applied to each bipolar channel within each selected 5-min data interval. We calculated the HFO rate for each bipolar channel of each patient by dividing the number of detected HFO on each channel by the duration of the analysed EEG recording, resulting in the unit HFO/min. For modelling purposes, we used the HFO rate detected in each 5-min data interval on the channel with the highest HFO rate during the recording night.

We controlled for the clinical plausibility of scalp HFO rate distributions by comparing the localisation of the channel with the highest HFO rate with the localisation of spikes in scalp EEG and focal lesions in MRI.

The software for the detection of HFO is freely available at the GitHub repository (https://github.com/ZurichNCH/Automatic-High-Frequency-Oscillation-Detector). Further available data and codes are indexed on our website https://hfozuri.ch

2.4 Modelling approach

To address the hypothesis that scalp HFO rates are modulated by sleep homeostasis, presenting with the highest rates in N3 sleep and decreasing with accumulated time in sleep, we fitted the HFO measurements to mathematical models. We modelled the HFO rate as a Poisson process, based on the methodology previously applied for HFO analysis in invasive EEG (von Ellenrieder et al., 2017). We assumed that HFO events are not overlapping and that time intervals between consecutive events are statistically independent (von Ellenrieder et al., 2017; Nagasawa et al., 2012; Nonoda et al., 2016).

Primary variables of interest affecting HFO rates were the current sleep stage (NREM N2, N3, REM), represented as a Boolean variable, and the time spent in sleep, determined as the elapsed time expressed in hours from the first sleep stage until waking up in the morning. We further included the delta band and the sigma band activity, estimated as the root-mean-square value of the bandpass filtered signal in the 0.5–4 Hz and the 10–16 Hz band during each 5-min data interval. The delta band and sigma band activity were calculated for the scalp channels F3–C4, F4–C4 (von Ellenrieder et al., 2017), averaged, and then normalised to have zero mean and unit variance for each analysed sleep stage and patient.

In a first step, to establish the best-performing Poisson process model explaining the modulation of scalp HFO rates by sleep homeostasis with the highest possible accuracy and generalizability, we employed a comparison between Poisson process models considering 15 different combinations of the explanatory variables (current sleep stage, time spent in sleep, delta band activity, sigma band activity). We estimated the mean HFO rate for every 5-min data interval as a function of these variables to provide statistical evidence for their contribution to the fluctuation of HFO rates during whole-night sleep. We considered the mean HFO rate calculated over the total analysed period and relative variations of the mean HFO rate as determined by the variables in the model. We used the Akaike Information Criterion (AIC) for model comparison (Burnham and Anderson, 2002) since its value indicates both the goodness-of-fit and the complexity of the model. The respective coefficients and the AIC values for all 15 models are given in Supplementary Table 1. Two models can be considered significantly different with 95% probability when the difference in the respective AIC values exceeds 6.

In a second step, to assess the performance and determine the generalizability of the Poisson process model considering our relatively small sample size, 1) we employed a comparison between the Poisson process model and the simplest model, i.e., the constant rate model, and 2) we applied a leave-one out cross-validation procedure. For cross-validation, we used all but one patient from our cohort to estimate the model coefficients and tested the result on this one patient, then repeated this procedure for all patients. We then compared the Poisson process model including all patient data for training (Poisson process train) with the Poisson process model cross-validating the model on the left-out patient (Poisson process test). The comparison of the predicted HFO rate in the 5-min data intervals, as provided by the Poisson process train model, the Constant rate model, and the Poisson process test model is illustrated by Supplementary Table 2, and Supplementary Fig. 1.

2.5 Test-retest reliability

To evaluate the reproducibility of scalp HFO detection, i.e. to investigate whether channel-wise HFO rates are consistent among different data intervals for each patient, we applied the test-retest reliability methodology as described in our previous work (Fedele et al., 2017). In short, we first calculated the normalised scalar products of HFO event vectors across different data intervals to depict the actual distribution and then created a null distribution from scalar products of HFO event vectors with permutated channel order. We report the mean number of scalar products with higher values than the 97.5th percentile of the null distribution, thus giving an estimation for the consistency of detected HFO rates in the analysed data intervals compared to randomised data.

For this analysis step, we constructed 10- and 15-min data intervals for each patient and for each sleep stage by concatenating consecutive 5-min data intervals throughout the whole-night EEG recording. To rule out a bias in our analysis arising from this specific data interval selection, we additionally constructed 10- and 15-min data intervals for each patient and for each sleep stage 1) by concatenating shuffled, i.e., randomly selected 5-min data intervals throughout the whole-night EEG recording, and, 2) by concatenating 5-min data intervals originating only from the beginning or only from the end of the whole-night EEG recording. We then compared the test-retest reliability of HFO detection 1) between consecutive and shuffled data intervals, and 2) between data intervals originating only from the beginning or only from the end of the whole-night EEG recording.

2.6 Statistics

We calculate the mean HFO rate over all recording intervals of each patient. Across patients, we describe distributions by their median and their interquartile range (iqr). To compare these distributions, we used non-parametric statistics. We also used the Wilcoxon signed-rank tests to compare the reliability values between sleep stages and data intervals of 5-, 10-, and 15-min duration. To quantify correlations, we used Spearman’s rank correlation. Statistical significance was established at p < 0.05.
3 Results

3.1 Patient characteristics, total length of sleep recordings, and HFO rate

We included 16 patients (4 female) with focal structural epilepsy (Table 1). The median age at the time of the whole-night EEG recording was 7.6 y (range 2.2–17.4 y). Aetiologies included focal cortical dysplasia in 8 cases, perinatal or childhood stroke in 3 cases, and single cases of diffuse glioma, hypothalamic hamartoma, Rasmussen’s encephalitis, hippocampal sclerosis, and hemiconvulsion-hemiplegia-epilepsy syndrome. The localisation of the presumed epileptogenic zone was frontal in 4 cases, temporal in 3, frontocentral in 2, parietal and temporoparietooccipital in one case each, and hemispheric in the remaining 5 cases.

We analysed 4735 min of EEG data, including 2480 min of N2, 1095 min of N3, and 1160 min of REM sleep. In total, we detected 5632 HFO: 2513 in N2, 1984 in N3 sleep, and 1135 in REM sleep. The median length of analysed data per patient was 132.5 min (iqr 90), with a median of 293.5 (iqr 363) detected HFO per patient.

The median length of analysed data per patient was 305 min (iqr 56), with a median of 52.2 (iqr 18.5) detected HFO during N2, 18.5 (iqr 18.5) during N3, and 18.5 (iqr 19.6) during REM sleep.

The median length of analysed data per patient was 132.5 min (iqr 95), for N2, 65 min (iqr 37.5) for N3, and 72.5 min (iqr 45) for REM sleep.

3.2. The scalp HFO rate is higher in N3 than in N2 or REM sleep

The mean HFO rate over all data intervals (NREM N2 + N3, REM) varied widely between patients (median 0.9 HFO/min, iqr 1.8), Table 1, Fig. 1A). The mean HFO rate was lower for N2 (median 0.3 HFO/min, iqr 1.7) and REM (median 0.6 HFO/min, iqr 1.2), and higher for N3 sleep (median 1.2 HFO/min, iqr 1.9) (Table 1). N3 rates were significantly higher than N2 rates (Wilcoxon signed rank test, p = 0.034, z = 2.12), but no significant difference was found between N2 and REM or N3 and REM rates (Wilcoxon signed rank test, p = 0.650, z = 0.25; p = 0.177, z = 1.35).

Across all patients, the mean HFO rates in N3 (median 23.3%, iqr 52.2) were higher while the mean HFO rates in N2 (median –22.8%, iqr 42.9) and REM (median –12.8%, iqr 77.1) were lower than the mean HFO rate of the patient (Fig. 1B). Supplementary Fig. 2 presents the spatial distribution of HFO rates in N2, N3 and REM sleep, with higher HFO rates in N3 compared to N2 and REM sleep for Patient 2.

3.3 The scalp HFO rate is higher in the first sleep cycle

In a first step, to explore the effect of time spent in sleep on the HFO rate through the different sleep stages, we used a simple Poisson process model including only the two explanatory variables of current sleep stage and time spent in sleep (Supplementary Table 1, Model 8). According to this simple model, the mean HFO rate decreased with time spent in sleep with a relative median rate change of –18.9% (iqr 29.9) per hour in N3 sleep compared to –3.5% (iqr 16.2) per hour in N2, and –4.8% (iqr 30.7) per hour in REM sleep (Fig. 1C).

In a second step, to assess the modulation of scalp HFO rates by sleep homeostasis with the highest possible accuracy and generalizability, we used the best Poisson process model including all four explanatory variables (Supplementary Table 1, Model 15). The coefficients of the relative HFO rate variation for all four included explanatory variables (current sleep stage, time spent in sleep, delta band activity, sigma band activity), as estimated by the best Poisson process model, are given in Table 2, in line with the methodology previously applied for HFO analysis in invasive EEG (von Ellenrieder et al., 2017), for reasons of comparability. In this table, units correspond to percentages for all explanatory variables of the Poisson process model, except for time spent in sleep, where units correspond to percentage per hour. According to the best model, the mean HFO rate decreased with time spent in sleep by –5.7%, –0.8%, and –6.9% per hour in N2, N3, and REM sleep.

Fig. 2 presents the hypnogram of Patient 2 with the respective delta- and sigma band activity and the HFO rate during NREM N2 and N3, and REM sleep stages, including both the measured rate and the rate estimated by the best Poisson process model. The figure illustrates the decrease of the HFO rate with time spent in sleep for Patient 2.

3.4. The scalp HFO rate increases with higher delta- and sigma band activity in N3 sleep

Delta and sigma band activity showed the expected distribution across sleep stages: N3 sleep was associated with the highest delta band activity (N3 median 45.0 \( \mu V \), iqr 25.5; N2 median 23.0 \( \mu V \), iqr 18.5; REM median 18.5 \( \mu V \), iqr 19.6), whilst sigma band activity was highest in N2 (N2 median 4.9 \( \mu V \), iqr 1.3; N3 median 4.7 \( \mu V \),...
The mean HFO rates is higher in N3 and decreases with time spent in sleep. The violin plot shows the median (white circle) and the interquartile range (black perpendicular line) of each distribution. A) HFO rate averaged over all N2, N3, and REM intervals for each patient. B) Compared to the mean HFO rate shown in panel A, the mean HFO rate is lower for N2 and REM intervals and higher for N3 intervals. C) The Poisson process model containing the sleep stage and time spent in sleep indicates that the HFO rate decreases per hour spent in sleep (N2 median −5.7%, N3 median −0.8%, REM −6.9% per hour). HFO: high frequency oscillations; REM: rapid eye movement.

### Table 2

| Explanatory Variable                      | N2              | N3              | REM             |
|-------------------------------------------|-----------------|-----------------|-----------------|
| Current sleep stage                       | −17.2           | 37.4            | −5.3            |
| Time spent in sleep                       | −5.7            | −0.8            | −6.9            |
| Delta band activity                       | 8.9             | 26.6            | −14.4           |
| Sigma band activity                       | 8.9             | 8.2             | 10.8            |

The table considers all four explanatory variables of the best Poisson process model: current sleep stage, time spent in sleep, delta band activity, sigma band activity. Units correspond to percentages for all explanatory variables of the Poisson process model, except for time spent in sleep time, where units correspond to percentage per hour. The coefficients determine how each explanatory variable will alter the HFO rate for the specific N2, N3, or REM interval. For example, an increase in delta band activity by one standard deviation during an N3 sleep interval corresponds to a 26.6% increase in HFO rate compared to the mean HFO rate for each patient. HFO: high frequency oscillations; REM: rapid eye movement; h: hour; std: standard deviation.

We calculated the test-retest reliability of HFO detection in 5-, 10-, and 15-min data intervals, with significant improvement in reliability when increasing the analysed data interval length from 5 to 10 min (Wilcoxon signed-rank, \( p = 0.004, z = 2.9 \)), but not when increasing from 10 to 15 min (Wilcoxon signed-rank, \( p = 0.879, z = 0.1 \)). For N2 sleep, the median reliability of HFO detection increased from 27% (iqr 68%) to 45% (iqr 73%) to 50% (iqr 67%) for 5-, 10-, and 15-min data intervals, with significant improvement in reliability when increasing the analysed data interval length from 5 to 10 min (Wilcoxon signed-rank, \( p < 0.001, z = 3.3 \)) but not from 10 to 15 min (Wilcoxon signed-rank, \( p = 0.054, z = 2.0 \)). For REM sleep, the median reliability of HFO detection increased from 35% (iqr 36%) to 54% (iqr 48%) to 50% (iqr 60%) for 5-, 10-, and 15-min data intervals, with significant improvement in reliability when increasing the analysed data interval length from 5 to 10 min (Wilcoxon signed-rank, \( p = 0.009, z = 2.6 \)) but not from 10 to 15 min (Wilcoxon signed-rank, \( p = 0.289, z = 1.1 \)).

It should be noted that there was no significant difference (Supplementary Table 3) between the test-retest reliability of HFO detection for 10- and 15-min data intervals constructed by concatenating consecutive (Fig. 3) and shuffled data intervals (Supplementary Fig. 3) during a whole-night EEG recording. However, the test-retest reliability of HFO detection was higher for 10- and 15-min data intervals originating from the beginning than for those originating from the end of the whole-night EEG recording, as illustrated in Supplementary Fig. 4.

Across all sleep stages (N2, N3, REM) and data interval lengths (5–15 min), we established a strong positive correlation between the HFO rates and the test-retest reliability of HFO detection (Spearman’s rank correlation, \( p < 0.05 \)). Nevertheless, the analysis of 10-min data intervals of N3 sleep provided considerably higher reliability than the analysis of shorter (5-min) data intervals.

### 4 Discussion

Electrophysiological markers of pathological epileptic brain activity show distinct dynamics associated with sleep stages and amount of sleep. This study demonstrates that scalp HFO in paediatric focal epilepsy change throughout whole-night sleep EEG recordings. To our knowledge, we are the first to determine the most sensitive time window in terms of sleep stage, cycle, and data interval length to ensure the quality and reproducibility of scalp HFO detection in paediatric epilepsy. We provide evidence that
the first N3 sleep stage during a whole-night scalp EEG recording yields the highest HFO rates of the whole night recording. We demonstrate that reliable measures of HFO detection can be achieved in 10-min data intervals of N3 sleep, with higher HFO rates correlating with higher reliability values. Our observations enable selecting appropriate data intervals for stable HFO estimates in the first step towards their implementation as an epilepsy biomarker in a clinical setting.

4.1 Scalp HFO rates are higher in N3 sleep

Scalp HFO rates were significantly higher in N3 sleep than N2 and REM sleep in our study, in line with the significantly higher spike rates occurring in N3 sleep compared to other vigilance states (Ng and Pavlova, 2013). We may hypothesise that the higher scalp HFO and spike rates in N3 sleep are determined by the same neuronal processes that determine the decrease of spontaneous firing rates of cortical neurons with sleep (Vyazovskiy et al., 2009).

Moreover, the effects of sleep stage on HFO rates in the scalp EEG of children with focal epilepsy reported here not only confirm previous observations deriving from the invasive EEG of adult patients (Al-Bakri et al., 2018; von Ellenrieder et al., 2017) but also extend these observations to a more accessible EEG modality and a much younger age group. Our study further demonstrates that including data from N3 sleep will increase the sensitivity of HFO detection because of the higher HFO rate in this sleep stage. This increased sensitivity is crucial for using HFO rate as a novel biomarker for epilepsy in the real-world clinical setting.

Finally, the remarkably high HFO rates in N3 sleep may be at least partly attributed to higher synchronicity levels in this sleep stage. This neuronal synchronisation results in the increased slope of slow-wave activity observed in N3 sleep and the amplitude of high-frequency activity. This state results in a higher phase-amplitude coupling between high (gamma, ripple) and low (theta or lower) frequencies in this sleep stage (Amiri et al., 2016). Our study replicated the strong positive covariation of HFO rate with delta band activity in N3 sleep, with less consistent effects in REM sleep. Previous reports have highlighted that delta band activity is less robustly associated with slow-wave amplitude in REM sleep, suggesting that the observed association between HFO rates and REM delta band activity is driven by a process distinct from the coupling of slow-wave amplitude and HFO rate previously

![Fig. 2. Hypnogram of a four-year-old patient with posterior right temporal lobe epilepsy. A) We present the hypnogram (dark blue line), the delta- and sigma band activity (light blue and black line), the measured HFO rate (green dots) and the modelled HFO rate (red dots) in N2, N3, and REM sleep as a function of time spent in sleep. The measured HFO rate is highest in N3 sleep and decreases with time spent in sleep. Each dot corresponds to a 5-min data interval. B) In the zoomed part of the hypnogram for the N3 sleep stage, we show how the 5-min data intervals are selected and then concatenated into 10-, and 15-min data intervals. Arrows mark the start and end of a concatenated interval, while the dashed part is not included in the analysis. HFO: high frequency oscillations; REM: rapid eye movement.](image-url)
reported in NREM sleep. Similar results have previously been reported based on invasive EEG recordings (von Ellenrieder et al., 2017; Nonoda et al., 2016).

4.2 Scalp HFO rate is higher in the first sleep cycle

The first N3 sleep stage during a whole-night scalp EEG recording in our study yielded the highest HFO rate, thus constituting the most sensitive time window for analysing HFO in paediatric epilepsy. Our findings are in line with the previously reported decrease of HFO rate with accumulated time in sleep in the invasive EEG of adults with focal epilepsy undergoing presurgical evaluation (von Ellenrieder et al., 2017), and confirm that the first sleep cycle is best suitable for studying HFO, irrespective of patient age and EEG modality.

The decrease of HFO rate with the accumulated time spent in sleep, in addition to its change with the different sleep stages, may be partly explained by the sleep-homeostatic changes in delta power showing a steady decline across the recording night (Riedner et al., 2007). This observation supports the notion that synchronisation, most pronounced during NREM slow-wave sleep, may be crucial for HFO generation (von Ellenrieder et al., 2017), and confirm that the first sleep cycle is best suitable for studying HFO, irrespective of patient age and EEG modality.

4.3 N3 data intervals of at least 10 min are required for scalp HFO analysis

To confirm the reproducibility and establish the reliability of our scalp HFO detection, we performed a test-retest analysis, as previously developed by our group (Fedele et al., 2017), investigating the spatial profile of HFO rates across several EEG data intervals from each patient.

We showed that, while for higher HFO rates, such as those encountered at the beginning of the night, reasonably high reliability is reached even when using only 5-min data intervals, for lower HFO rates, such as those encountered in the later part of the night, longer data intervals are indispensable. Overall, HFO detection is more reliable for patients with high HFO rates, whereas longer data intervals ensure higher reliability values for high HFO rates and even for lower HFO rates.

Based on the findings from our cohort, we suggest that N3 data intervals of 10 minutes are required for scalp HFO analysis.
especially for non-structural/genetic epilepsy, or for different underlying aetiologies of focal structural epilepsy.

4.4 Future directions

HFO in focal epilepsy have been shown to be modulated by sleep in all brain regions except for the frontal lobe in an invasive EEG study, including ten patients with frontal lobe coverage (Dümpelmann et al., 2015). To investigate the effect of lobar localisation of the HFO generator, larger cohort sizes than reported here are required.

Sleep stage has been demonstrated to determine the extent of HFO spread in an invasive EEG study focusing on the effect of sleep homeostasis on HFO (von Ellenrieder et al., 2017). Whilst standard EEG does not allow for evaluating such localised effects, similar questions may be investigated using high-density scalp EEG in the future (Fan et al., 2021). This outlook is especially relevant for the clinical setting, as high-density scalp EEG can be readily implemented in clinical epilepsy units (Zelmann et al., 2014).

Different lesion types present different degrees of intrinsic epileptogenicity and higher HFO rates have been previously shown to mirror the higher epileptogenicity of specific FCD subtypes in intracranial EEG recordings (Kerber et al., 2013). However, the heterogeneity of our focal epilepsy cohort has prevented us from addressing the impact of lesions and their aetiologies on delta and sigma band activities and, potentially, on HFO rates. To investigate the effect of aetiology on HFO rates and their interaction with sleep homeostasis, larger cohort sizes with higher homogeneity are required.

Our finding of relatively lower HFO rates in REM sleep and higher in NREM N3 sleep supports the notion that HFO generation is correlated with the mechanisms generating slow waves and is thus homeostatically regulated. The decrease of HFO rate with delta band activity that is, in turn, decreasing with time spent in sleep, as is also the case for slow wave activity, primary marker of the homeostatic process, leaves room for the interpretation of HFO generation in the light of the two-process model of sleep regulation (Borbély, 1982; Borbély et al., 2016). Although the analysis of our findings within the framework of the two-process model is impeded by the lack of data on sleep behaviour outside of the recording duration (e.g., naps, sleep deprivation etc), this direction could be pursued in future datasets from long-term EEG recordings performed over several days and nights for presurgical evaluation in epilepsy surgery candidates, and where sleep deprivation may be applied to activate and thus capture epileptic seizures.

5 Conclusion

Our study provides a robust and reliable framework for scalp HFO detection that may facilitate their implementation as an EEG biomarker in paediatric epilepsy. The first N3 sleep stage during a whole-night scalp EEG recording yields the highest HFO rates, thus corresponding to the most sensitive time window for HFO analysis. Reliable measures of HFO detection can be achieved by the analysis of at least 10-min N3 sleep data intervals. These carefully selected intervals may suffice for clinically meaningful results, particularly for higher HFO rates. Non-invasively detected scalp HFO may prove an essential resource for clinical assessment in a broad population of children affected by epilepsy.

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.

Acknowledgments

We thank B. Alessandri, C. Carosio, L. Glaser, P. Hieber, and G. Selmin for their assistance with EEG recordings and data analysis. We thank the Swiss National Science Foundation [CRSK–3_190895 to G.R. and J.S.] and the Swiss League Against Epilepsy [Research Recognition Award to G.R.] for funding. The funders had no role in the design or analysis of the study.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2021.12.013.

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Acknowledgments

We thank B. Alessandri, C. Carosio, L. Glaser, P. Hieber, and G. Selmin for their assistance with EEG recordings and data analysis. We thank the Swiss National Science Foundation [CRSK–3_190895 to G.R. and J.S.] and the Swiss League Against Epilepsy [Research Recognition Award to G.R.] for funding. The funders had no role in the design or analysis of the study.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinph.2021.12.013.
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