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RESEARCH ARTICLE

Prolongation of cortical sleep spindles during hippocampal interictal epileptiform discharges in epilepsy patients

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Abstract

Objective: Memory deficits are frequent among patients with epilepsies affecting the temporal lobe. Hippocampal interictal epileptic discharges (hIEDs), the presumed epileptic exaggeration of sharp wave-ripples (SWRs), are known to contribute to memory dysfunction, but the potential underlying mechanism is unknown. The precise temporal coordination between hippocampal SWRs and corticothalamic spindles during sleep is critical for memory consolidation. Moreover, previous investigation indicated that hIEDs induce neocortical spindlelike oscillation. In the present study, we aimed to assess the influence of hIEDs on neocortical spindles.

Methods: We analyzed the spindle characteristics (duration, amplitude, frequency) of 21 epilepsy patients implanted with foramen ovale (FO) electrodes during a whole night sleep. Scalp sleep spindles were categorized based on their temporal relationship to hIEDs detected on the FO electrodes. Three groups were created: (1) spindles coinciding with hIEDs, (2) spindles "induced" by hIEDs, and (3) spindles without hIED co-occurrence.

Results: We found that spindles co-occurring with hIEDs had altered characteristics in all measured properties, lasted longer by 126 ± 48 ms (mean ± SD), and had higher amplitude by 3.4 ± 3.2 μV, and their frequency range shifted toward the higher frequencies within the 13–15-Hz range. Also, hIED-induced spindles had identical oscillatory properties to spindles without any temporal relationships with hIEDs. In more than half of our subjects, clear temporal coherence was revealed between hIEDs and spindles, but the direction of the coupling was patient-specific.

Significance: We investigated the effect of hippocampal IEDs on neocortical spindle activity and found spindle alterations in cases of spindle–hIED co-occurrence, but not in cases of hIED-initiated spindles. We propose that this is a marker of a pathologic process, where IEDs may have direct effect on spindle generation. It could mark a potential mechanism whereby IEDs disrupt memory

processes, and also provide a potential therapeutic target to treat memory disturbances in epilepsy.

KEYWORDS
epilepsy, hippocampus, interictal epileptiform discharges, sleep spindle

1 | INTRODUCTION

Cognitive deficits, mostly affecting memory functions, are common among chronic epilepsy patients. Traditionally, interictal epileptiform discharges (IEDs) were considered to cause only transient deficits,[1-3] but there is growing evidence that they are related to long-term and possibly progressive memory disturbances.[4-8] As yet, it remains unknown how IEDs are indicative of such impairment and what processes of the physiological memory network they disrupt.

The key electrophysiological markers of declarative memory network during non-rapid eye movement (NREM) sleep are hippocampal sharp wave-ripples (SWRs),[9] thalamocortical spindles,[10] and the <1-Hz cortical slow oscillation.[11] The hierarchical nesting of these three oscillations generates a synchrony over widespread regions in brain activity, which is hypothesized to facilitate information exchange between the hippocampus and the cortex, while the newly encoded memories (temporarily stored in hippocampal networks) could be redistributed to neocortical networks for long-term storage.[12-14]

Sleep spindles are hallmarks of NREM sleep stage 2 (N2), and are characterized by waxing and waning oscillations with a 10–16-Hz frequency and with ≥.5-s duration.[15] They play a critical role in memory consolidation and brain plasticity.[16,17] Although spindle characteristics such as spindle duration, amplitude, and frequency are traitlike individual features, their modifications were reported in a variety of physiological and pathological conditions: learning,[18] aging,[19-21] developmental, neurodegenerative, and psychiatric diseases,[22-25] and last but not least, epilepsy.[26]

Hippocampal IEDs (hIEDs) are pathologically intensive synchronous excitatory events considered to be the pathological exaggeration of SWRs, the epileptic spike-pathological ripple complex.[9] SWRs' conversion to hIEDs and pathologic high-frequency ripples was evidenced in a rat kindling model.[27] Similarly to SWRs, hIEDs also show a temporal relationship with sleep spindles.[28] Moreover, previous studies including human and animal data, indicated that hIEDs can induce spindlelike oscillations in the frontal regions even in awake state, which pathological coupling was found to be related to spatial memory impairments.[27]

Key Points

- In two thirds of our subjects, temporal coherence was revealed between hippocampal IEDs and sleep spindles, but the direction of the coupling was patient-specific
- Spindles co-occurring with hIEDs had altered characteristics, lasted longer, and had higher amplitude, and their frequency range shifted toward the higher frequencies
- hIED-induced spindles had identical oscillatory properties to spindles without any temporal relationships with hIEDs
- Spindle–hIED co-occurrence might be a marker of a pathologic process, whereby hIEDs have direct effect on spindle generation

We aimed to study the temporal relationship between hIEDs and neocortical sleep spindles and the potential alterations in spindle characteristics in relation with this interaction in refractory patients examined for temporal lobe foci implanted with parahippocampal foramen ovale (FO) electrodes.

2 | MATERIALS AND METHODS

2.1 | Subjects

We retrospectively analyzed the records of 54 patients who underwent intracranial electroencephalographic (EEG) investigation with implanted FO electrodes as a routine phase 2 procedure of their presurgical evaluation between 2004 and 2018 at the National Institute of Psychiatry and Neurology, later National Institute of Clinical Neurosciences, Budapest, Hungary. All patients were selected for electrode implantation entirely based on clinical grounds independent of any scientific considerations. We only considered patients with 10–50/min mesiotemporal IED rate, to provide sufficient events in all spindle groups in all patients, and to reduce the likelihood of occasional coincidence due to abundant amount

of IEDs. We further selected the patients for single-peaked IED waveforms, to ensure reproducible timing of IED peaks, and a minimum of 200 detected spindles in N2 per night. Also, patients with more than one seizure per night were excluded.

Altogether, 33 patients were excluded; eight patients had poor data quality, six patients had multiple peaked IED waveforms, five patients had low N2 spindle number over the night, and 17 patients had out of range IED density (eight cases low and nine cases high). Seven patients did not have any nights with fewer than two seizures. The final sample consisted of 21 patients; nine were female, mean age was 35.7 ± 9.9 years (range = 18–60), the average duration of epilepsy was 12.7 ± 10.4 years (range = 1–42). Fourteen patients had temporal and seven patients extratemporal pathology. Detailed patient characteristics are summarized in Table 1. Informed consent for the video monitoring, scalp and FO recordings, and the use of the data for research purposes was obtained from all patients. The study was approved by the ethics committee of the National Institute of Clinical and Neurosciences, Budapest, Hungary (IRB 8/2016).

2.2 | EEG recording and processing

All recordings were acquired using the actual version of Brain Quick System (Micromed; with System 2, System Plus, and System Evolution software). Sampling rate was 256 (in two patients), 512 (in five patients), or 1024 Hz (in 14 patients). EEG signals were recorded to a vertex reference. Recordings were amplified and digitized with 22-bit resolution. Scalp electrodes were placed using the International 10–20 system[29] extended with anterior temporal electrodes (F9/10, Ft9/10, T9/10, Tp9/10).[30] Four- or six-contact FO leads were used that were 1 mm in diameter (AdTech). FO signals were analyzed in bipolar montage of the adjacent contacts, resulting in three to five bipolar channels bilaterally.[31]

One full night sleep was selected for analysis. The first postimplant night was excluded, and the one with the longest sleep period without epileptic seizure was selected. In cases without seizure-free nights, a single seizure was allowed and the seizure period starting 30 min before and terminating 30 min after the seizure was excluded. Patients with multiple seizures per night were excluded.

Sleep stages and artifacts were scored visually for 30-s epochs according to standard criteria[32] by an expert trained in sleep research. We included all the artifact-free N2 epochs in the further analyses.

2.3 | Detection of spindle and IED activity

Offline analysis was performed with MATLAB (v9.3.0.713579, R2017b, MathWorks), using the EEGlab toolbox[33] and custom developed functions.

2.3.1 | Spindle detection and analysis

A previously established method[18,34] adopted from Crowley et al. was used.[35] EEG signals were offline rereferenced to averaged Tp9 and Tp10 to construct the mean mastoid reference. Sleep spindle detection was performed separately on scalp EEG channels F3, F4, P3, and P4. The raw EEG was band-pass filtered between 11 and 16 Hz using a zero phase shift Hamming window based filter. A spindle event was detected wherever the filtered signal exceeded an individually calculated amplitude and a fixed duration criterion. Amplitude threshold was set to three times the SD of the amplitude across N2 sleep epochs. Spindle onset was defined by the time point where the envelope signal (magnitude of the Hilbert transform) crossed the threshold, and offset when the signal fell under the threshold. Detections with duration < .5 s were rejected. The spindle detection method was validated previously.[18] We calculated the following parameters for all the patients and for all four scalp electrodes: (1) the mean spindle duration, (2) the mean maximal amplitude (defined by the mean maximum of the envelopes of filtered EEG signals over the detected spindles); and (3) the power spectral density. The peak frequency was defined as the frequency corresponding to the maximum spectral power.

2.3.2 | IED detection

IED detection was performed on all of the bipolar FO and the selected four scalp (F3, F4, P3, and P4) channels. The channels with the highest IED density at each side were chosen for the analyses. In case of multiple channels with the same density, the channel with higher voltage was considered. For IED detection, an amplitude threshold method was used. FO channels were high-pass filtered (5 Hz, two-way least-squares finite impulse response filtering). Potential IEDs were identified if the filtered signal exceeded the mean of the envelope of the filtered signal by 3 SD. The largest absolute voltage during the event was considered as the peak of the IED. The spindle detection approach was validated against visual analysis by an expert. Visual detection was carried out blinded. Agreement

TABLE 1 Demographic, clinical, and anatomical characterization of subjects

Patients	Age, years	Sex	Etiology	Handedness	Age at onset of epilepsy, years	Epilepsy duration, years	Seizure onset zone	Antiseizure medication
1	37	Male	HS (left)	R	32	5	Left medial temporal	LEV, LTG, CBZ
2	31	Male	Parahippocampal GG (left)	R	1	30	Left basal temporal	TPM, CBZ
3	27	Female	Insular LGA (right)	L	26	1	Right insular	GBP, RUF
4	30	Male	Normal	R	21	9	Bitemporal	LEV, OXC, ZNS
5	60	Female	Viral (?) encephalitis	L	33	27	Bitemporal	LTG, OXC, LEV
6	41	Male	HS (left)	R	22	19	Left temporal	TPM, LTG, CLN
7	52	Male	Amygdalar lesion (left; posttraumatic)	R	33	19	Left mesial temporal	CBZ, LCM, LEV
8	29	Male	HS (bilateral; postencephalitis)	R	.1	28.9	Bitemporal	PHB, CLN
9	42	Female	HS (right)	R	0.2	41.8	Right mesial temporal	OXC
10	30	Female	HS (right)	R	26	4	Left temporal	LEV, LTG
11	34	Male	Normal	L	17	17	Bitemporal	LTG, PHB
12	26	Female	Temporal FCD (left)	R	3	23	Left frontobasal	CBZ, LEV
13	35	Female	Mitochondrial encephalomyopathy/ multifocal lesions	R	34	1	Multifocal	OXC, CLN, LTG
14	40	Female	Parahippocampal ODG (left)	R	32	8	Left temporal	TPM, ZNS, BDZ, CBZ
15	32	Female	Frontal FCD (left)	R	12	20	Left frontal	LEV, CBZ, ZNS, CLN
16	18	Male	Normal	R	13	5	Bitemporal	CBZ, LTG, BDZ
17	42	Male	Normal	R	22	20	Bitemporal	LEV, VPA
18	41	Male	HS (right)	R	10	31	Bitemporal	CBZ, LTG, BDZ
19	27	Male	HS (right)	R	25	2	Right mesial temporal	VPA, LTG, BDZ
20	27	Female	Temporal FCD (right)	R	9	18	Bitemporal	VPA, LTG
21	49	Male	HS (right)	L	12	37	Bitemporal	VPA, CBZ, CLN

Abbreviations: BDZ, benzodiazepine; CBZ, carbamazepine; CLN, clonazepam; FCD, focal cortical dysplasia; GBP, gabapentin; GG, ganglioglioma; HS, hippocampal sclerosis; L, left; LCM, lacosamide; LEV, levetiracetam; LGA, low-grade astrocytoma; LTG, lamotrigine; ODG, oligodendroglioma; OXC, oxcarbazepine; PHB, phenytoin; R, right; RUF, rufinamide; TPM, topiramate; VPA, valproate; ZNS, zonisamide.

between the two methods resulted in on average 89.3% (±7.1%) for true positive detections and 17.1% (±8.3%) for false positives during NREM sleep.

2.4 | Spindle categories

Spindles were categorized based on their temporal relationship to hIEDs. Three groups were created: (1) spindles coinciding with hIEDs (spike-spindle [SSP]), defined by an ipsilateral hIED peak falling between the onset and offset of the spindle; (2) spindles induced by hIEDs (induced spindle [ISP]), when an ipsilateral hIED peak was detected within 1 s before spindle onset; and (3) no spike spindles (NSP), when no ipsilateral hIED detection was in the time range between 1 s before onset and offset. See Figure 1 for illustration. If multiple criteria (1 and 2) were true, or a scalp detected IED coincided with the spindle, the event was rejected.

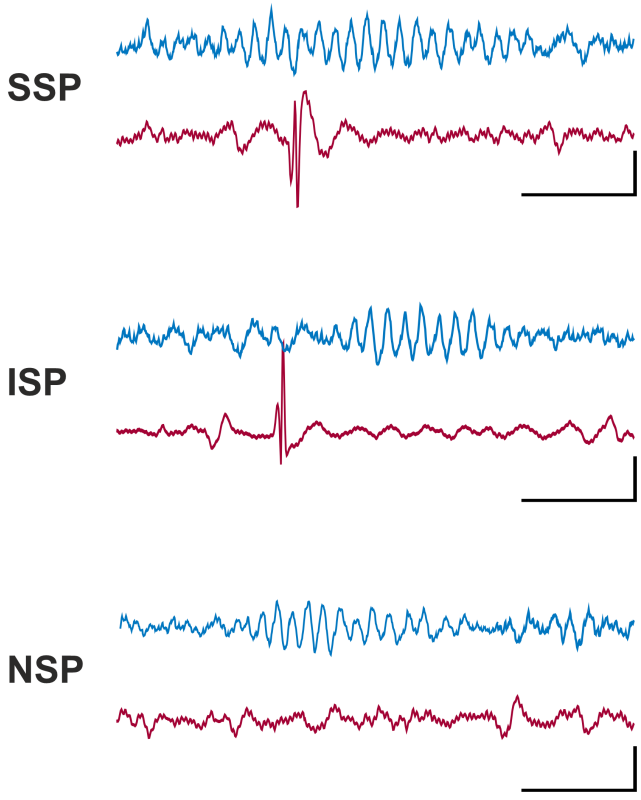

FIGURE 1 Experimental design. Sleep spindles registered from scalp electrodes (F3, F4, P3, P4; blue; scale bar = 50 μV, 500 ms) and hippocampal interictal epileptic discharges simultaneously registered from foramen ovale electrode (red; scale bar = 100 μV, 500 ms) in Patient 1 are shown. Examples of spindle types: SSP = scalp spindles (blue line) that co-occurred with hippocampal interictal epileptiform discharges (IEDs; red line), ISP = scalp spindles that occurred within 1 s after a hippocampal IED, NSP = scalp spindles with no temporal connection with hippocampal IEDs

2.5 | Antiseizure medications

Antiseizure medications (ASMs) were categorized based on their main pharmacodynamics into the following groups: (1) Na channel inhibitors (lamotrigine, carbamazepine, lacosamide, oxcarbazepine, rufinamide), (2) Ca channel inhibitors (gabapentin, zonisamide), (3) γ-aminobutyric acid type A ($GABA_A$) receptor modulators (phenobarbital, clonazepam), (4) synaptic vesicle 2A modulator (levetiracetam), and (5) polypharmacological mechanism (valproate, topiramate). To investigate the possible pharmacological effects on spindle characteristics, we analyzed spindle duration and spindle amplitude between patients who use a given type of ASM and those who do not. This comparison was performed for all the abovementioned medication groups separately.

2.6 | Cross-correlation

We computed cross- or autocorrelograms between hIED peak and spindle onset times. IED times were taken as cross-correlogram references. We applied the MATLAB function "crosscorrelogram," which uses a lagged cross-correlation method that is appropriate for discrete time series.[36]

2.7 | Neuropsychological assessment

Of 21 patients, 19 had detailed neuropsychological examination in the 4-month period (from 2 months prior to 2 months after) of the video-EEG monitoring as part of the presurgical examination. According to memory investigation, the following tests were performed with the 19 patients: Rey Auditory Verbal Learning Test (RAVLT; $n = 15$), Rey–Osterrieth Complex Figure test (ROCF; $n = 17$), Location Learning Test ($n = 15$), and Verbal Reasoning Test ($n = 13$). Of these tests, we chose to assess the RAVLT and ROCF delayed recall, because they reflect memory consolidation, storing, and retrieval. Delayed recall scores were correlated with the ratio of SSPs (ratio of SSPs to all other spindles).

2.8 | Statistical analysis

Statistical analysis was performed using custom MATLAB code. Differences between the spindle groups were analyzed by the nonparametric Wilcoxon signed rank test. Durations, amplitudes, and peak frequency for the spindle groups were extracted from each other, whereas in the null hypotheses, we assumed that these

differences do not differ from zero. Significance level was set to $p = .05$, and the Bonferroni method was applied to correct for multiple comparisons; the corrected p value was .05/12 = .00416 (rounded to the fifth decimal). Twelve statistical comparisons were performed: four electrode sites × three groups.

The power changes in the spindle spectra were assessed using a permutation test. Five thousand random samples were generated to test for statistically significant differences in each .5-Hz wide frequency bin between the two compared spindle groups.[37] Two-sided paired t-tests were used in each frequency bin; t-values were set against the values of the randomly shuffled values from both spindle groups. Benjamini–Hochberg procedure for false discovery rate (FDR) was applied to control for multiple comparisons by adjusting the p-values obtained for all frequency bins. Separate tests were performed at each derivation.

Due to small sample sizes, the Kruskal–Wallis test was used to compare ASM groups.

For cross-correlation statistics, we used the "xcorrpvalue" MATLAB function that yields p-values of significance of the maximum cross-correlation vector using a permutation test with random vectors. If the random vector fraction was <.0005, then cross correlation was considered significant.[38]

For group comparisons of spindle characteristics between patient groups of different epilepsy variables and cross-correlogram "types," we used Mann–Whitney or Kruskal–Wallis tests, depending on the nature of the data analyzed.

3 | RESULTS

3.1 | Sleep spindle and hIED characteristics

A total of 3695.2 min of artifact-free N2 sleep was analyzed (range = 72.6–328.3 min per subject). For detailed characteristics, see Table 2. The duration, amplitude, and frequency of the spindles in all recording sites agreed with previous reports.[19,38]

The median hIED rate across all subjects on FO channels was 31.9 ± 17.01/min (range = 14.2–48.9) for the left side and 21.89 ± 5.81/min (range = 11.1–41.3) for the right side.

Spindles were assigned to three categories (NSP, ISP, SSP). All patients expressed all three spindle types, but with differing proportions. For detailed description of the individual count of the spindle types, see Table S1.

3.1.1 | Duration

Spindles coupled with hIEDs (SSP) had longer duration at all recording sites compared to spindles induced by IEDs (ISP vs. SSP; for all electrodes $p < 10^{-5}$) and spindles without IED connection (NSP vs. SSP; for all electrodes $p < 10^{-5}$), whereas duration of ISPs and NSPs did not differ (for all electrodes, $p > .1$). Mean duration differences between SSPs and NSPs were 134 ± 62, 129 ± 46, 139 ± 52, and 104 ± 35 ms at F3, F4, P3, and P4 locations, respectively. Results are presented in Figure 2 and detailed in the extended data Table S2.

To determine whether timing of hIEDs within the spindle span has implications on spindle duration, we divided the duration of SSPs into three equal parts and compared spindle durations when hIED coincided in the first or the last third. No differences were found (for all electrodes, $p > .1$).

We also aimed to assess whether the prolongation of spindles varied between the frontal and the parietal electrodes. Duration differences of SSP and NSP spindles were compared between the ipsilateral frontal and parietal electrodes (F3-P3, F4-P4). No significant differences were revealed (F3 vs. P3, $p = .121$; F4 vs. P4, $p = .601$).

3.1.2 | Amplitude

SSPs had higher maximal amplitude over the other groups (SSP vs. ISP for all electrodes, $p < 10^{-4}$; SSP vs. NSP for all electrodes, $p < 10^{-4}$). There was no amplitude difference between ISP and NSP (for all electrodes, $p > .1$). Results are presented in Figure 2 and detailed in Table S3.

TABLE 2 Sleep spindle characteristics at each electrode

Characteristic	F3, mean ± SD	F4, mean ± SD	P3, mean ± SD	P4, mean ± SD
Density, /min	6.76 ± 3.56	6.24 ± 3.91	7.08 ± 4.01	5.92 ± 4.52
Duration, ms	837 ± 291	832 ± 98	829 ± 224	817 ± 107
Maximal amplitude, μV	43.16 ± 9.813	42.91 ± 10.022	39.43 ± 11.813	38.76 ± 13. 041
Frequency, Hz	12.276 ± 2.977	12.601 ± .924	13.240 ± 5.207	13.556 ± 1.039

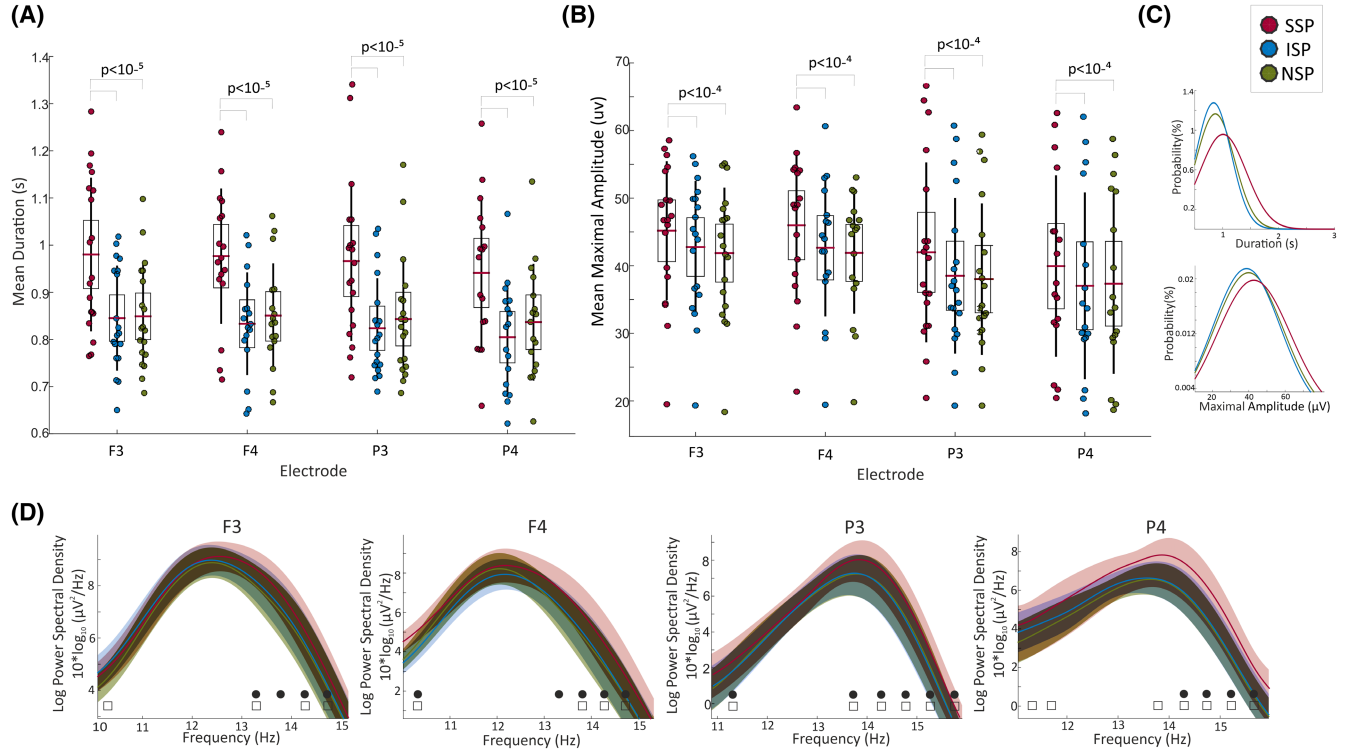

FIGURE 2 Characteristics of the spindle types. SSP = scalp spindles that co-occurred with hippocampal interictal epileptiform discharges (IEDs; red), ISP = scalp spindles that occurred within 1 s after a hippocampal IED (blue), NSP = scalp spindles with no temporal connection with hippocampal IEDs (green). (A) Spindle duration (horizontal lines = mean, boxes = 95% confidence interval, vertical bold lines = SD). Note that SSP had a longer duration at each recording site, whereas ISP had a duration similar to NSP. Numbers of events included in the analysis: SSP (F3) = 6453, (F4) = 5312, (P3) = 6678, (P4) = 5453; ISP (F3) = 7589, (F4) = 6134, (P3) = 7312, (P4) = 6478; NSP (F3) = 10 865, (F4) = 9876, (P3) = 11 567, (P4) = 9893. For detailed statistics, see Table S2. (B) Spindle maximum amplitudes. Note that SSP had the highest maximum amplitude. Numbers of events: SSP (F3) = 6453, (F4) = 5312, (P3) = 6678, (P4) = 5453; ISP (F3) = 7589, (F4) = 6134, (P3) = 7312, (P4) = 6478; NSP (F3) = 10 865, (F4) = 9876, (P3) = 11 567, (P4) = 9893. For detailed statistics, see Table S3. (C) Probability distribution of durations (upper panel) and maximum amplitudes (lower panel) across spindle groups at all electrodes. (D) Differences in power spectra (mean ± SEM). SSP exhibited a relative increase in spectral power within the higher sigma frequency ranges. Dots indicate significant frequency bins corresponding to corrected $p < .05$ between SSP and ISP; squares indicate significant frequency bins corresponding to corrected $p < .05$ between SSP and NSP

Also, amplitude difference did not vary between the frontal and parietal electrodes (F3 vs. P3, $p = .629$; F4 vs. P4, $p = .795$).

3.1.3 | Frequency

The peak frequency was not different among the spindle groups; however, the maximal power of the peak frequency was higher in SSP (SSP vs. ISP, all corrected $p < .001$; SSP vs. NSP, all corrected $p < .001$) at all electrodes.

We aimed to further specify the difference, so we investigated the power spectrum at all frequency bins in all spindle groups. The SSP group exhibited a relative increase in spectral power within the higher sigma frequency ranges compared to other spindle groups; frequency bins remaining significant after FDR correction were 12.5–15.5 Hz in the frontal electrode sites and 13.5–15.5 Hz in the parietal electrode sites (SSP vs. NSP for all electrodes, $p < 10^{-5}$; SSP vs. ISP for all electrodes, $p < 10^{-5}$; presented in Figure 2).

3.2 | Temporal relationship between sleep spindles and hIEDs

To study the temporal relationship between spindles and hIEDs, we computed cross-correlations between hIED peak occurrences and spindle onsets. (For individual cross-correlation results of each patient, see Table S4). In 11 patients (52%), we found significant cross-correlation, but the direction of this coherence was of two kinds. In eight (39%) patients, the hIEDs tended to precede the spindles, with an average time offset of 450 ± 92 ms for the frontal electrodes and 378 ± 99 ms for the parietal ones. In contrast, in three patients (14%),

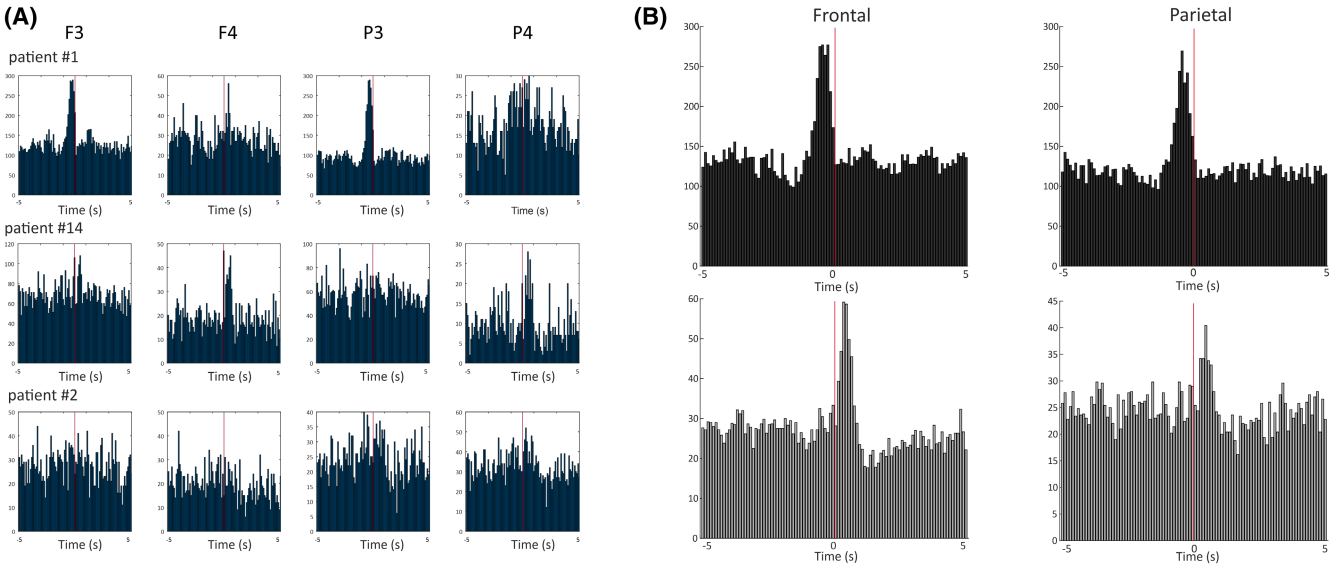

FIGURE 3 Cross-correlation of hippocampal interictal epileptiform discharges (hIEDs) and scalp spindle onsets. Red lines indicate peak of hippocampal IEDs. (A) Examples from patients with high and low correlations. In Patient 1, spindles preceded IEDs. Numbers of events: IEDs: right = 6939, left = 1717; spindles: F3 = 1514, F4 = 1233, P3 = 1135, P4 = 818. In Patient 14, IEDs preceded spindles. Numbers of events: IEDs: right = 1571, left = 6503; spindles: F3 = 785, F4 = 854, P3 = 691, P4 = 449. In Patient 2, we found no correlation. Numbers of events: IEDs: right = 1253, left = 4280; spindles: F3 = 765, F4 = 1869, P3 = 744, P4 = 929. (B) Averaged cross-correlogram of patients with significant correlations. Upper panel shows patients with significant correlation where spindles preceded IEDs ($n = 3$; numbers of events: IED = 31 463; spindle frontal = 9651, parietal = 9804; $p_{\text{frontal}} = 6.345 \times 10^{-5}$, lag = −430 ms; $p_{\text{parietal}} = 1.567 \times 10^{-5}$, lag = −410 ms). Lower panel shows patients with significant correlation where IEDs preceded spindles ($n = 8$; numbers of events: IED = 57 484; spindle: frontal = 20 013, parietal = 16 347; $p_{\text{frontal}} = 7.934 \times 10^{-8}$, lag = 410 ms; $p_{\text{parietal}} = 2.676 \times 10^{-6}$, lag = 430 ms). For individual cross-correlation of hIEDs and spindle onsets of each patient, see Table S4

hIEDs tended to follow the spindles with similar timing; the average time offset was 462 ± 268 ms for the frontal and 400 ± 100 ms for the parietal electrodes. (For examples of individual cross-correlograms and also for averaged cross-correlograms of each group, see Figure 3). Next, we investigated the effect of temporal correlation differences on spindle characteristics. We found no duration or amplitude difference among spindle groups when patients were allocated into three groups based on the temporal coherence (patients with no significant correlation between hIEDs and spindles; patients with significant coherence, where spindles tended to preceded hIEDs; and patients with significant coherence, where spindles tended to follow hIEDs). For detailed data, see Table 3.

3.3 | Epilepsy variables

Differences in spindle characteristics were considered when patients were divided into groups based on epilepsy variables. The mean duration and mean maximal amplitude among spindle groups did not vary when patients were divided into groups based on epilepsy duration and hippocampal lesion. For detailed data, see Table 3.

3.4 | Antiseizure medications

Spindle duration and maximal amplitude in patients taking Na channel inhibitors ($n = 18$), Ca channel inhibitors ($n = 4$), $GABA_A$ modulators ($n = 10$), levetiracetam ($n = 7$), and drugs with polypharmacological mechanisms ($n = 7$), did not differ significantly (all p values > .1) from other patients.

3.5 | Neuropsychological assessment

Correlations between the ratio of SSPs and the delayed recall score on the RAVLT and ROCF were analyzed separately for all four electrodes: F3, F4, P3, and P4. Because the distribution of SSP ratio values deviated significantly from normal distribution, Spearman correlations were used with statistical control of years of intractable seizures. The correlation between SSP ratio and ROCF delayed recall score reached a statistical tendency level (Spearman $r = -.49$, $n = 15$, $p = .076$) at P3 electrode. Correlations

TABLE 3 Effect of cross-correlogram "types" and epilepsy variables on spindle characteristics

Characteristic	*n*	SSP, mean ± SD	ISP, mean ± SD	NSP, mean ± SD
Temporal relationship				
Mean duration				
No temporal connection	9	.968 ± .172	.851 ± .127	.830 ± .114
Temporal connection with spindles preceding hIEDs	3	.953 ± .186	.857 ± .149	.857 ± .128
Temporal connection with spindles following hIEDs	8	.996 ± .118	.861 ± .067	.866 ± .086
Kruskal–Wallis, *p*		.113	.451	.378
Mean maximal amplitude				
No temporal connection	9	43.107 ± 13.533	39.750 ± 12.594	40.585 ± 13.022
Temporal connection with spindles preceding hIEDs	3	44.405 ± 8.656	41.386 ± 7.533	42.397 ± 7.323
Temporal connection with spindles following hIEDs	8	45.49 ± 3.718	41.132 ± 2.795	42.926 ± 3.167
Kruskal–Wallis, *p*		.287	.734	.685
Epilepsy variables				
Mean duration				
Hippocampal lesions	12	.952 ± .134	.830 ± .091	.833 ± .106
No hippocampal lesions	9	1.017 ± .195	.875 ± .134	.862 ± .119
Mann–Whitney *p*, *U*		.395, 91	.600, 87	.600, 87
Epilepsy duration < 10 years	8	.972 ± .206	.865 ± .159	.841 ± .134
Epilepsy duration > 10 years	13	1.007 ± .187	.866 ± .123	.853 ± .113
Mann–Whitney *p*, *U*		.866, 101	.942, 100	.642, 104
Mean maximal amplitude				
Hippocampal lesions	12	44.694 ± 8.652	41.491 ± 7.992	42.838 ± 8.715
No hippocampal lesions	9	45.853 ± 12.543	42.393 ± 11.925	43.342 ± 11.431
Mann–Whitney *p*, *U*		.778, 84	.657, 86	.840, 83
Epilepsy duration < 10 years	8	41.415 ± 12.606	38.185 ± 11.083	39.533 ± 11.254
Epilepsy duration > 10 years	13	45.695 ± 12.047	42.124 ± 11.352	42.831 ± 11.321
Mann–Whitney *p*, *U*		.447, 107	.575, 105	.575, 105

Abbreviations: hIED, hippocampal interictal epileptiform discharge; ISP, induced spindles; NSP, no spike spindles; SSP, spike-spindles.

between SSR and RAVLT scores did not reach statistical significance ($p < .05$) or tendency level ($p < .1$) at any electrodes.

4 | DISCUSSION

Our study is the first to report differences in spindle characteristics due to hIEDs in humans. Spindles co-occurring with hIEDs lasted longer and had higher amplitude, and their frequency range shifted toward the higher frequencies. Previous studies including human and animal data indicated that hIEDs can induce spindles in the frontal regions even in awake state.[27] To study this effect, we also analyzed those spindles that were preceded by hIEDs within a 1-s time window. We found that these induced spindles lacked the same difference and were statistically similar to spindles without temporal correlation to hIEDs.

Spindles temporally coupled with hIEDs lasted approximately 140 ms longer. It is well known that spindles are generated in the thalamus, through alternating excitation of thalamocortical and nucleus reticularis thalami (nRT) neurons. Studies on spindle termination in rodents showed that although nRT firing dropped sharply before the end of spindles, the length itself depended on the magnitude of nRT cell activation at the onset.[45] The authors suggested an unspecified ongoing network activation being responsible for this effect. As regards the effect of IEDs on spindle length, two possible mechanisms might be invoked. The first assumes that an ongoing network

activation favors both long spindle emergence and IED generation, whereas the second hypothesis states that occasional co-occurrence of the IEDs and spindles affects the current network's state that is responsible for nRT activation level, resulting in a pathologically prolonged spindle. In the abovementioned investigation, it was not tested whether altering the network state during an ongoing spindle can have any impact on spindle length. Because we found variable interaction between IED and spindle timing that had no further effect on spindle length, we hypothesized the existence of different network mechanisms separately responsible for timing and length of the spindles, and IED generation seemed to interact independently with them.

In addition to the length of sleep spindles, the amplitude of spindle oscillations was increased in the case of IED co-occurring spindles. Previous reports found higher spindle amplitudes to be associated with the epileptic hemisphere in patients with focal epilepsies,[26] whereas no lateralization was observed in idiopathic generalized epilepsy patients. Our findings could account for this observation. In addition, larger amplitude sleep spindles were shown to recruit more widespread cortical areas, thus being more global[38,46]; therefore, changes in amplitude might account for altered function.

We also found enhanced power in the higher frequency bins corresponding to higher sigma range, whereas the peak frequency was not changed. Enhanced spectral power of 12.5–15.5 Hz was observed on both the frontal and parietal electrodes, although SSPs in P3/P4 showed more prominent increase than SSPs in F3/F4. Although duration and amplitude changes were similar between the frontal and parietal regions, frequency changes might indicate some preferential regional coupling, even if not significantly. Physiological spindles can be divided into slow (11.0–12.75 Hz) and fast (12.5–14.5 Hz) categories,[38,47] among which the slow spindles were biased more to the frontally, whereas fast ones predominated rather in the centroparietal areas and were suggested to be engaged more in memory processes.[48,49] Our findings imply that parietal fast spindles interact more with hIEDs than the frontal, slower spindles. Previous studies[14] reported that posterior hippocampal spindle-ripples are connected to rather temporoparietal sleep spindle activity, showing strong interaction of these regions. The relatively posterior position of FO electrodes along the hippocampal axis supports this observation that posterior hIEDs interact more with the parietal fast spindles.

As mentioned earlier, these changes in spindle properties were not seen in induced spindles. Gelinas et al.[27] found hIEDs induced frontal lobe spindling, and in concordance with our findings, these induced spindles exhibited similar oscillatory characteristics with physiological frontal lobe spindles, including waveforms, duration, and peak frequency. It should be mentioned that the interpretation of IEDs inducing spindles was indicated by an observed increase in spindle occurrence after IEDs. In our study, spindles were considered "hIED induced" when they were preceded by hIEDs, but we have no evidentiary data that ISPs were induced by hippocampal epileptic discharges.

To find further evidence of this spindle inducing effect, we analyzed the spindle onset timings and found variable relationships. In more than half of our subjects, clear temporal coherence was found between hIEDs and cortical spindles, but this attribution was patient-specific. In 52% of the patients, hIEDs tended to precede the spindles with a ~ .4-s time frame, and another 14% showed the opposite relationship with the same time window. For the previous group, our findings are consistent with a prior study confirming a similar correlation of temporal IEDs with frontal spindles,[27] but in reverse, spindles preceding IEDs were not reported before. We revealed no preferential regional coupling; it could be present at frontal and also at parietal electrodes. This also contradicts the previous report, where the substantial correlation was found in the frontal lobe. Different anatomical relationships have been identified for epileptic foci and spindles.[39,40] It is possible the percentage of patients with different types of correlation is affected by other epileptic activity that is not detected with the current methods. Also, Gelinas et al.[27] detected spindles on intracranial electrodes in four patients, whereas we used scalp electrodes for the same purpose in a higher number of cases. In contrast with scalp detected spindles, which are highly synchronous and widely distributed, intracranially or intracortically detected spindles are known to be more asynchronous and focal.[40-43] Also, the intracranial involvement during scalp spindles has no consistent pattern.[44] Further investigations are needed to characterize the temporal relationship of hippocampal spikes and cortical spindles. However, irrespective of the existence and also of the direction of this temporal relationship, only the spindles co-occurring with hIEDs had altered characteristics.

The question has emerged, whether such relatively subtle changes in spindle characteristics correspond to a difference in the function. Because spindle parameters are known to show strong intraindividual stability,[50,51] they are the most heritable EEG signatures,[52] and even minor alterations in their characteristics frequently correlate with decrease in cognitive abilities and neuropsychiatric disorders,[22-25] we proposed these alterations to have a pathological nature, and to be the consequence of the epileptic circuitry.

We investigated whether altered spindle characteristics via hIED co-occurrence has any impact on memory consolidation. A tendency level correlation was found

between memory consolidation in terms of delayed recall and the ratio of SSPs in the left parietal region. This might indicate that an increase in the number of spike spindles may decrease memory consolidation and thus memory performance. However, this finding is limited by the small sample size. Further studies with larger sample sizes will be needed to corroborate this finding, and to control for other clinical factors. Also, this correlation does not reflect the immediate effect of altered spindling on memory, because the memory testing sessions and the electrophysiological recordings were days or weeks apart.

Analyzing the differences in spindle characteristics across medication groups, we did not find any medication effect on spindles. However, this finding is limited by the small sample size; considering that spindle groups were compared within subjects in our study, it is unlikely that our major findings are biased by medication effects.

Based on the result that all measured properties changed in hIED co-occurring spindles, we concluded that these alterations are the consequence of the pathologic process induced by the IEDs themselves, which may have a direct compromising effect on spindle generation. This could mark a potential mechanism whereby IEDs disrupt memory processes and also provide a potential therapeutic target to treat memory disturbances in epilepsy. Further studies are needed to test the cognitive effect of pathologically affected spindle production, and to test the direct influence of hippocampal activations on spindle generation.

Limitations of our study are the relatively small sample size, the heterogeneous epilepsy etiologies and localizations of epileptogenic lesions, and also the pharmacotherapy variations. Also, we investigated patients with a limited IED density; therefore, we excluded 33 patients out of 54, which may entail some bias.

AUTHOR CONTRIBUTIONS

Anna Sákovics: Conceptualization (equal), methodology (equal), writing–original draft (lead), formal analysis (equal), visualization (lead), writing–review and editing (supporting). Gábor Csukly: Formal analysis (equal), writing–review and editing (supporting). Csaba Borbély: Investigation (equal). Márta Virág: Investigation (equal). Anna Kelemen: Investigation (equal). Róbert Bódizs: Methodology (supporting), writing–original draft (supporting), writing–review and editing (supporting). Loránd Erőss: Investigation (equal), writing–review and editing (supporting). Dániel Fabó: Conceptualization (equal), methodology (equal), writing–original draft (supporting), formal analysis (supporting), visualization (supporting), writing–review and editing (lead).

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CONFLICT OF INTEREST

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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