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Brief Communication

# Prolonged and short epileptiform discharges have an opposite relationship with the sleep–wake cycle in patients with JME: Implications for EEG recording protocols

Francesco Turco [a,b,*], Filippo Sean Giorgi [a,b], Michelangelo Maestri [a], Riccardo Morganti [c], Alessandro Benedetto [b], Chiara Milano [a], Chiara Pizzanelli [a], Danilo Menicucci [d], Angelo Gemignani [d], Francesco Fornai [b,e], Gabriele Siciliano [a,b], Enrica Bonanni [a,b]

[a] Department of Clinical and Experimental Medicine, Neurological Clinic, University of Pisa, Italy
[b] Department of Translational Research and of New Surgical and Medical Technologies, University of Pisa, Pisa, Italy
[c] Section of Statistics, University Hospital, Pisa, Italy
[d] Department of Surgical, Medical and Molecular Pathology and Critical Care Medicine, University of Pisa, Italy
[e] IRCCS Neuromed, Pozzilli, Italy

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ABSTRACT

In a recent study, we found that during 20.55 ± 1.60 h of artifact-free ambulatory EEG recordings, epileptiform discharges (EDs) longer than 2.68 s occurred exclusively in patients with Juvenile Myoclonic Epilepsy (JME) who experienced seizure recurrence within a year after the EEG. Here we expanded this analysis, exploring whether long EDs (>2.68 s), and short ones, were uniformly distributed during the day. Lastly, we evaluated the temporal distribution of seizure relapses.

By Friedman test, we demonstrated that hourly frequencies of both short and long EDs were dependent on the hours of day and sleep–wake cycle factors, with an opposite trend. Short EDs were found mostly during the night (with two peaks at 1 AM and 6 AM), and sleep, dropping at the wake onset ($p < 0.001$). Conversely, long EDs surged at the wake onset (0.001), remaining frequent during the whole wake period, when compared to sleep ($p = 0.002$). Of note, this latter pattern mirrored that of seizures, which occurred exclusively during the wake period, and in 9 out of 13 cases at the wake onset.

We therefore suggested that short and long EDs could reflect distinct pathophysiological phenomena. Extended wake EEG recordings, possibly including the awakening, could be extremely useful in clinical practice, as well as in further studies, with the ambitious goal of predicting seizure recurrences.


## 1. Introduction

We recently demonstrated that, in patients with Juvenile Myoclonic Epilepsy (JME), the maximum length of generalized epileptiform discharges (EDs) recorded during prolonged ambulatory EEG (paEEG) predicts motor seizure recurrences. More precisely, a cut-off of 2.68 s discriminated between cases (i.e., patients with seizure relapse within one year after paEEG) and controls (i.e., persons with JME not experiencing seizures within 1 year after paEEG) with high accuracy; none of the patients in the control group exhibited EDs longer than 2.68 s in paEEG covering the whole sleep–wake cycle, and 13 out of 14 patients in the case group had at least one ED longer than 2.68 s [1]. Incidentally, our findings are in line with the current arbitrary cutoff of 2 or 3 s, which is used by many epileptologists to distinguish the interictal EDs from the subclinical seizures in absence syndrome [2]. We therefore hypothesize that long and short EDs could reflect distinct pathophysiological phenomena.

In genetic generalized epilepsy (GGE), seizures occur mostly during the wake period [3]. In JME, they have a typical circadian distribution with a peak in the morning, immediately after the awakening [4]. Paradoxically, the highest number of EDs in GGE is observed during the night, peaking before the awakening, and dropping after sleep offset [5]. Of note, in GGE, not only ED frequency, but also ED length vary throughout the 24 h, reaching a maximum during the wake period [6]. Moreover, in patients with JME, very prolonged EDs tend to occur in the morning, immediately after the awakening [7].

* Corresponding author at: Department of Clinical and Experimental Medicine, Neurological Clinic, University of Pisa, Italy.
*E-mail address:* francesco.turco@phd.unipi.it (F. Turco).

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There is a growing interest in rhythmicity in epilepsy. Data from focal epilepsy syndromes suggest that the knowledge of the rhythmicity of interictal EDs and clinical event occurrence may improve our ability to forecast seizures (see Starling et al. [8] for a review). Regarding GGE, circadian and sleep–wake patterns of ED occurrence have been evaluated systematically only by two groups [9,10], but no relation with seizure recurrence was highlighted. Benefits for patients with epilepsy would come from deeper understanding of the relationships between seizures, epilepsy, and the EEG features that enable seizure prediction [11].

Here, we aimed at verifying whether short and long EDs, and seizures, have a different distribution in relation to the time of the day and the sleep–wake cycle in JME. This might also have an immediate clinical impact, helping to disclose whether shorter EEG recording protocols may be as effective as prolonged EEG ones to detect long EDs.

## 2. Methods

We analyzed the same paEEG recordings from our recently published study [1]; data were retrospective and included recordings from patients with JME whose clinical outcome is known. The study had been approved by our Institutional Review Board and all patients had given their written consent to have their clinical data analyzed for research purpose. All patients maintained the same anti-seizure medication (ASM) regimen (dosage and type) throughout the whole period from the EEG recording up to the outcome events (seizure recurrence for cases, at least 1-year seizure freedom for controls). The database included information on all ED length and time of occurrence (EDs were defined as generalized spikes, polyspikes, spike-and-waves and polyspike-and-waves); sleep stages were scored according to AASM scoring criteria [12], and periods of large artifacts were excluded from the analysis. For further details, see Turco et al. [1].

In the present study, we classified each ED as short or long, according to the cutoff previously identified (2.68 s), and we calculated their hourly frequency during the 24 h and across the sleep–wake cycle.

To study the impact of sleep–wake cycle on EDs and seizures, we split wake period in three partitions: "wake offset" as one continuous hour of wake preceding any period of sleep (either during afternoon or night, including short nap), "wake onset" as one continuous hour of wake following any period of sleep, and "wake" as the remaining periods of wakefulness (i.e., excluding "wake offset" and "wake onset"). We checked if the factors "hour of day" and "sleep–wake cycle phase" had an impact on short and long ED frequency. Since this latter showed a strong variability, with many patients having very few, or no EDs during prolonged periods of recordings, we adopted non-parametric statistical methods.

All the analyses were performed with custom-made MATLAB (MATLAB, R2016B) scripts, employing the built-in statistical toolbox. The null hypothesis was rejected for $p < 0.05$.

## 3. Results

Detailed clinical features of the patients are reported in our previous work [1]. Since EDs longer than 2.68 s occurred only in the case group, we can refer to that paper for comparison between recording with or without long EDs. More in details, 11 recordings were free of any EDs (1 case, 11 controls), short EDs occurred in 21 recordings (13 cases, 8 controls), and both long and short EDs occurred in 13 recordings, all from the original cases group. Overall, short ED results are based on 20.55 ± 1.60 h and long EDs on 20,17 ± 1.73 h of artifact-free recordings.

Cases and controls did not differ in age, disease duration, time from the start of the first ASM regimen calculated at the time of EEG acquisition, the number and type of drug regimens previously tried and failed, and type of antiseizure medication regimen at the time of EEG recording. Briefly, both in the case and control groups there were a similar proportion of (1) patients who were on their first therapy type, whose dosage was recently adjusted (cases = 3/14; controls = 4/18, $p = 0.96$), (2) women of childbearing potential, shifted from VPA to a new drug regimen (LEV or LTG), even if VPA was effective (cases = 9; controls = 6; $p = 0.08$), and (3) patients who had been put on a second or third drug regimen after one or multiple therapeutical failures (cases = 2; controls = 8; $p = 0.07$).

A standard EEG recording with activation methods performed immediately before the paEEG showed a photoparoxysmal response in only one patient from the control group, and in none from the case group, without any statistical difference (see Turco et al Epilepsia, Table 1). When considering how many patients have ever displayed photosensitivity on the standard EEG during their whole clinical history, we found that there were not statistically significant differences between the two groups (incidence of photoparoxysmal response in case group = 21.43%; in control group = 33.33%; $p$ value computed by Fisher exact test = 0.811). Based on the analysis of paEEG recordings used in the present analysis, two parameters providing information on sleep stability, i.e., total sleep time during the whole day and Sleep Efficiency of nocturnal sleep, could be inferred in the two groups; both parameters were not statistically significantly different between groups (total sleep time during the whole day was: cases 452.63 ± 76.60, controls 454.12 ± 64.17; $t$-test $p$ value = 0.957. Sleep Efficiency of the nocturnal sleep: cases 92.96 ± 5.61, controls 91.98 ± 26.06; $t$-test $p$ value = 0.788).

### 3.1. Long and short ED variation across the 24 h

Visual inspection of ED's hourly frequency suggested that short EDs were more frequent during the night, peaking at midnight, and a steady decreasing toward the morning, with a deep trough in late afternoon. Contrariwise, long EDs occurred during the day, peaking in the morning, and declining during the night, especially at 3AM, when they were not found in any recording (Fig. 1, left panel).

Hourly frequency of both short and long EDs were affected (short EDs $p < 0.001$; long EDs $p = 0.001$) by "hour of day" (within factor with 18 levels in the Friedman test). Hours from 9 AM to 2 PM were not included in this analysis, as data were not available for 4 or more subjects, due to (a) premature interruption of recording, or (b) large artifacts for more than 30 min per hour of recording.

Regarding short EDs, *post hoc* analysis based on Wilcoxon paired sample test with Bonferroni correction for multiple comparisons individuated a trough at 6 PM, which was significant when compared to 2 AM ($p = 0{,}022$), to 5 AM ($p = 0{,}021$), to 1 AM ($p = 0{,}013$), and to 6 AM ($p = 0{,}001$). Significant peaks were found at 1 AM and at 6 AM (1 AM when compared to 6 PM, to 5 PM -$p = 0{,}049$-, and to 8 PM -$p = 0{,}005$; 6 AM when compared to 6 PM, to 5 PM -$p = 0{,}001$-, to 8 PM -$p = 0{,}005$-, to 7 PM -$p = 0{,}007$-, to 10 PM -$p = 0{,}025$-). Concerning long EDs, there were significant peaks at 7 AM and 8 AM, and troughs at 0 AM, 1 AM, and 3 AM, but none of these survived the Bonferroni correction.

### 3.2. Long and short ED frequency across the sleep–wake cycle

Visual inspection of ED's hourly frequency suggested that short EDs had a "sleep" preference, dropping at the "wake onset", and remaining rare during the whole wake. Conversely, long EDs were

**Table 1**
Impact of the sleep–wake cycle on short and long temporal distribution: post hoc analysis with sleep as the reference.

| | Short EDs | | Long EDs | |
|---|---|---|---|---|
| | Median (IQR) | *p*-value | Median (IQR) | *p*-value |
| Sleep | 7.065 (1.978–13) | reference | 0.000(0.000–0.346) | reference |
| Wake onset | 0.333 (0.000–3.250) | <0.001 | 2.999(1.249–6.749) | 0.001 |
| Wake | 0.121 (0.000–1.207) | <0.001 | 0.305(0.062–1.836) | 0.568 |
| Wake offset | 0.000 (0.000–1.499) | <0.001 | 0.000(0.000–1.750 | 1.000 |

EDs = epileptiform discharges. IQR = interquartile ranges. *P*-values refers to Wilcoxon paired sample test with Bonferroni correction for multiple comparisons.

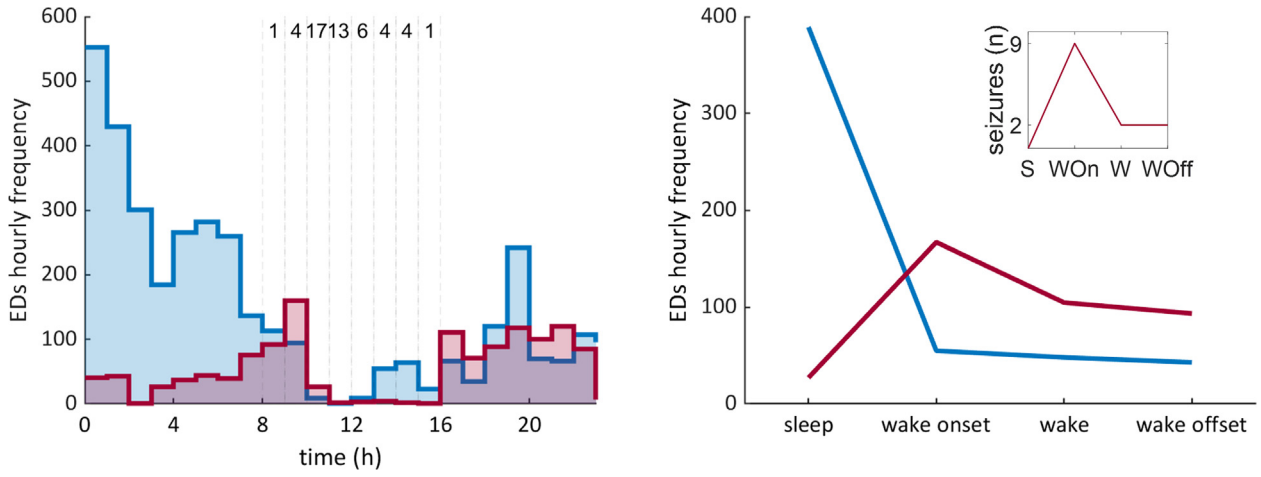


**Fig. 1.** Temporal patterns of occurrence of short (blue) and long (red) epileptiform discharges (EDs) across the 24 h (left) and the sleep–wake cycle (right). For visualization purposes, for both the panels, *Y* axis refers to the sum of all EDs occurred per hour from all the subjects. In the left panel, *X* axis = hours of day, and dashed lines and superimposed numbers display how many recordings are not available or are contaminated by artifacts lasting more than 30 min. In the right panel, in the small insert, is shown the distribution of seizure recurrences during the sleep–wake cycle (S = sleep, WOn = wake onset, W = wake, WOff = wake offset).

rare during "sleep", surged at "wake onset", and remained frequent during the whole wake period (see Fig. 1, right panel).

Both short and long EDs' hourly frequency were significantly ($p < 0.001$) affected by the sleep–wake cycle ("sleep–wake phase" factor with 4 levels used in the Friedman test). Accordingly, Wilcoxon paired test between the whole wake, including boundaries, and sleep, further demonstrated that short EDs tend to occur during sleep ($p < 0.001$), and long EDs during the wake ($p = 0.002$).

Table 1 reports the results of the post hoc analysis with "sleep" as the reference for both long and short EDs. Concerning long EDs, the peak at "wake onset" was significantly higher compared to "sleep" (see Table 1), and "wake onset" ($p = 0{,}005$), but not to "wake, excluding boundaries".

### 3.3. Time of seizure occurrence

For this analysis, we used the clinical outcome of 13 out of 14 cases of our previous work, as in one patients from the case group, the paEEG was free of any EDs (see Turco et al. [1]). For each patient, we considered only the first seizure occurring after the paEEG because, after that, following clinical practice, anti-seizure medication regimens were promptly changed. Multinomial test demonstrated that seizures were not uniformly distributed along the sleep–wake cycle ($p < 0.001$): seizures recurred at the "wake onset" in 9/13 cases, during "wake excluding boundaries" in 2/13 cases, and at "wake offset" in 2/13 cases, and never during sleep.

## 4. Discussion

The main result of this study is that short and long EDs have an opposite relationship with the sleep–wake cycle, the latter one reflecting that of seizures.

A limitation of this study is a relative lack of data from 9 a.m. to 2.pm. (see Result section and Fig. 1, left panel), which prevented us to further infer on the circadian rhythmicity of the EDs. However, we think this had a minor impact on the analysis of sleep–wake patterns, since (1) at least one hour of artifact-free EEG recording preceding the sleep onset, and following the sleep offset, was available for all the recordings, (2) All the sleep periods (including the afternoon nap) were recorded, and (3) most of the wake were recorded from each patient and was free of artifact.

Short EDs tend to occur during night-time and sleep, with two peaks at 1 AM and 6 AM. Conversely, they are sporadic during daytime and wake, as well as at wake boundaries (i.e., "wake onset" and "wake offset"), and their lowest frequency is observed at 6 PM. Interestingly, a study by Seneviratne et al. [9] found a peak of all ED frequency in GGE patients from 11 PM to 7 AM and a minimum at 6 PM.

Contrariwise, long EDs tend to occur during wake, with a surge at the wake onset, which is significant when compared to sleep, but not to other wake periods (i.e. "wake" and "wake offset"). Long EDs display a less clear relationship with the time of the day, compared to short EDs. Lack of statistical power could partially explain the absence of statistical significance for their peak in the morning

hours. Indeed, data on long EDs are based on fewer observations, since these are rarer than the short ones, occurring in only 13/32 against 18/32 recordings. Nevertheless, the inspection of global (Fig. 1, left panel), and individual (Turco et al., supportive information) [1] patterns of ED occurrence reveals that a large amount of long EDs occurs throughout the whole daytime. Long EDs that did not occur in the morning are partly found at the wake onset after brief period of sleep, partly random during wake. This pattern mirrors that of seizures reported by patients at follow-up, which occurred almost exclusively during the wake period with a predominance at the wake onset, in line with the literature on JME [13].

From another point of view, we individuated three patterns of daily ED distribution in patients with JME. Patients who will be seizure free for a long time could either have no EDs during the 24 h, or a large amount of short EDs occurring mostly during sleep; patients at risk of seizure recurrence have a large amount of short EDs occurring mostly during sleep and few to many long EDs occurring during the wake, predominantly at the wake onset.

We propose that prolonged EEG recording may be considered as the gold standard approach for disease monitoring in patients with JME. Indeed, shorter recording protocols would neglect a relevant proportion of long EDs, particularly when the assessment does not include the wake onset.

Future studies, hopefully in the context of a multicenter collaboration, could also allow a direct comparison of the cost-effectiveness of shorter protocol, such as sleep-deprived EEG, with the paEEG. The latter is known to have a high diagnostic yield, but its prognostic value has never been explored and recording protocols are not standardized in terms of length of the wake period recorded [14].

The duration of EDs occurring during sleep could be limited thanks to protective mechanisms such as K-complexes and sleep slow oscillations that have been associated with the breakdown of information processing in favor of sleep stability [15]. In this light, studying sleep in patients with JME remains of extreme interest. On the other hand, the mechanisms underlying the occurrence of longer EDs and seizures during the wake have been poorly investigated, and, the present paper might help to highlight this feature, fostering basic and translational research to further investigate those phenomena. Perturbational approaches employing the combination of Transcranial Magnetic Stimulation and EEG in healthy persons have demonstrated that cortical excitability increases during sleep, while cortico-cortical connectivity breaks down [16]. Visual Evoked Potentials (VEPs), performed during sleep, confirm that the sleeping brain is unable to effectively synchronize large neuronal populations in response to rapid sensory stimulation [17]. One could hypothesize that cortical excitability and the likelihood of short ED occurrence increase together during NREM sleep, but at the same time reduced cortico-cortical connectivity prevents the abnormal electrical activity to evolve into a prolonged epileptiform discharge or an epileptic seizure.

More in general, we suggest that in JME short and long EDs reflect distinct pathophysiological mechanisms that involve the abnormal recruitment of those circuits which are physiologically involved in sleep (as suggested by Beenhakker et al. [18]) and wake. The former might be involved in short interictal epileptiform discharge generation, the latter in the onset of long epileptiform discharges and seizures.

## 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.

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## Ethical Publication Statement

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.

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