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Interictal spikes and epileptic seizures: their relationship and underlying rhythmicity

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We report on a quantitative analysis of electrocorticography data from a study that acquired continuous ambulatory recordings in humans over extended periods of time. The objectives were to examine patterns of seizures and spontaneous interictal spikes, their relationship to each other, and the nature of periodic variation. The recorded data were originally acquired for the purpose of seizure prediction, and were subsequently analysed in further detail. A detection algorithm identified potential seizure activity and a template matched filter was used to locate spikes. Seizure events were confirmed manually and classified as either clinically correlated, electroencephalographically identical but not clinically correlated, or subclinical. We found that spike rate was significantly altered prior to seizure in 9 out of 15 subjects. Increased pre-ictal spike rate was linked to improved predictability; however, spike rate was also shown to decrease before seizure (in 6 out of the 9 subjects). The probability distribution of spikes and seizures were notably similar, i.e. at times of high seizure likelihood the probability of epileptic spiking also increased. Both spikes and seizures showed clear evidence of circadian regulation and, for some subjects, there were also longer term patterns visible over weeks to months. Patterns of spike and seizure occurrence were highly subject-specific. The pre-ictal decrease in spike rate is not consistent with spikes promoting seizures. However, the fact that spikes and seizures demonstrate similar probability distributions suggests they are not wholly independent processes. It is possible spikes actively inhibit seizures, or that a decreased spike rate is a secondary symptom of the brain approaching seizure. If spike rate is modulated by common regulatory factors as seizures then spikes may be useful biomarkers of cortical excitability.

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Introduction

Epilepsy is one of the most common severe neurological diseases, and is characterized by an increased likelihood for the brain to enter seizure states (Duncan *et al.*, 2006). A key goal in the treatment of epilepsy is to predict when seizures are likely to occur. Developing prediction strategies is extremely challenging due to the patient-specific causes of seizures, and the difficulty in obtaining long-term data. While much work is focused on activity at the level of individual neurons or interacting networks over short time periods, valuable insight about seizure dynamics may also be gained from studying long-term, higher-level statistics. The integration of all available information over a broad range of spatial and temporal scales is vital to achieving accurate seizure prediction.

Seizures are not sudden events, but may follow a build-up period where the brain's state is altered over the preceding hours to days (Litt *et al.*, 2001; Badawy *et al.*, 2009). Studies of inter-seizure interval distributions also suggest that past events influence the timing or dynamics of future events over periods of up to weeks and months (Osorio *et al.*, 2009; Cook *et al.*, 2014). For close to a century, it has been recognized that seizure occurrence demonstrates temporal patterns, following circadian and ultradian cycles (Langdon-Down and Russell Brain, 1929; Griffiths and Fox, 1938). However, surprisingly little is known about the cause of this oscillatory behaviour, in particular long-term rhythms that presumably have an underlying hormonal factor. While a slow build-up to seizure onset may blur the temporal precision of prediction algorithms, this long-range temporal dependency also provides a clear avenue for enhancing prediction using pattern recognition and long-term recordings.

Another challenge for seizure prediction is that seizures are difficult to define electroencephalographically. Seizures with clinical symptoms are often only a small proportion of all abnormal electrical activity in the brain, which includes subclinical seizures, interictal spikes, bursts and high-frequency oscillation. It remains unclear precisely how (or even if) seizures are related to this broader range of epileptic activity. Elucidating this relationship may provide valuable information for seizure prediction strategies. The current work addresses the relationship between seizures and interictal spikes.

Interictal spikes arise from the synchronous firing of a hyperexcitable population of neurons and are considered an abnormal electrical phenomenon. Spikes are associated with epilepsy (Ward, 1959; Ayala *et al.*, 1973; Schulze-Bonhage, 2011); however, there is conflicting evidence regarding the relationship between spiking and ictogenesis (Gotman, 1991; De Curtis and Avanzini, 2001; Avoli *et al.*, 2006). One hypothesis is that increased neural excitability promotes epileptic spikes, which may reach a critical spatial or temporal density and lead to seizure (Jensen and Yaari, 1988; De Curtis and Avanzini, 2001). Previous results have suggested there is a predictive or causal relationship between interictal spikes and ictogenesis (Ayala *et al.*, 1973; Avoli *et al.*, 2002; Uva *et al.*, 2005). However, there is relatively little experimental evidence supporting the conjecture that spikes precede ictal onset, in particular from studies based on human tissue recordings (De Curtis and Avanzini, 2001; Avoli *et al.*, 2006).

In contrast with the aforementioned studies, there are also reports showing that spike rate is largely unchanged or even reduced prior to seizures (Engel and Ackermann, 1980; Gotman and Marciani, 1985; Librizzi and de Curtis, 2003). This suggests that spikes may provide some protective benefit against seizures. The spike is often followed by a period of hyper-polarization, which may contribute to limiting the frequency of periodic interictal activity (Matsumoto and Marsan, 1964). Alternatively, spontaneous interictal discharge may provide regulatory, low-level excitation to forestall the transition to seizure (De Curtis and Avanzini, 2001; Avoli *et al.*, 2006). There may also be a secondary mechanism that influences patterns of both spikes and seizures.

The conflicting reports regarding the interaction between epileptic spikes and seizures indicates a complex relationship. This study investigates patterns of activity of seizures, spikes, and the relationship between the two. Data for the study were obtained from intracranial EEG from humans that were acquired during a clinical trial for an implantable, advance warning system for seizure (Cook *et al.*, 2013). The data were recorded over time periods ranging from 6 months to 2 years and covered tens to thousands of seizures per subject (both clinical and subclinical). This study represents the first analysis of interictal spike activity over such a long period in humans and the first opportunity to understand the relationship between interictal epileptic spikes and seizures.

Materials and methods

Data

For a more detailed account of the data collection procedures and demographics of the 15 subjects, the reader is referred to Cook *et al.* (2013). In short, two electrode arrays with a total of 16 platinum iridium contacts in moulded silicone carriers were implanted on the cortical surface. The arrays were placed over the region assumed to contain the epileptogenic zone based on prior imaging or clinical history. Electrode leads were tunnelled to an implanted telemetry unit located under the clavicle. The telemetry unit sampled 16 channels of EEG at a rate of 400 Hz that was wirelessly relayed to an external, portable personal advisory device. The personal advisory device stored the EEG for later analysis and was also capable of creating audio recordings that were used in subsequent seizure detection.

Seizure detection was initially undertaken by clinical staff then verified by expert investigators with the aid of the subjects' seizure diaries, device audio recordings, and a previously

validated automated seizure detection algorithm (Gardner *et al.*, 2006). Ictal events were classified as either being present in the EEG and associated with clinical symptoms (type 1), not clinically evident but located using the EEG recording and electroencephalographically indistinguishable from clinical seizures (type 2), or having no clinical correlation (either symptomatic or electrographic) but appearing as abnormal events in the EEG (type 3). Events that were clinically reported but not demonstrated in the EEG were discounted from the analysis. Every detected event was reviewed by study investigators in order to eliminate false positives.

Automated spike detection

There are many reported methods that are applied to the problem of automated spike detection in intracranial EEG data and no consensus on the most reliable method exists (Wilson and Emerson, 2002). In this study, a template matching algorithm was applied. The spike template was set to a fixed length of 0.3 s and the template window was advanced through the EEG by one sample (2.5 ms). The initial spike template was set for each subject by hand-selecting and averaging 10–20 spikes. New spikes could then be detected and added to the initial template.

The match between the template, x, and each 300 ms of intracranial EEG, y, was defined as the magnitude of the sample correlation, $|r_{xy}|$, where

$$r_{xy} = \frac{\sum_{i=1}^{n}(x_i - \overline{x})(y_i - \overline{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \overline{x})^2 \sum_{i=1}^{n}(y_i - y)^2}}$$

and n is the number of samples in the window ($n = 120$ for a sampling rate of 400 Hz).

To add to the initial, hand-selected template, a first pass over the data was performed at a high threshold ($r_{xy} = 0.96$ to 0.98 depending on the subject). The first pass was terminated when a final template containing 100 spikes was obtained. This process of creating a final template was repeated at semi-regular intervals of days to weeks (dependent on the size of the stored data epochs) throughout the trial, to capture long-term variation in spike shape. The final template was used in the detection algorithm. A spike was detected when $r_{xy} > 0.85$ and the spike amplitude exceeded the background mean by a sufficient amount. The background mean was defined as the channel average over the previous 1 s window. Candidate spikes were required to be within $\pm(0.5 \times$ final template height) of the background mean. The background threshold helped minimize the false positive rate due to artefact or other oscillations that contained smaller but similar shapes. A graphical representation of the detection algorithm is shown in Fig. 1. The figure shows that tuning the detection threshold gives the same output pattern but alters the balance of false positives and false negatives. There is no way to eliminate these errors, however, as this study was investigating long-term patterns of activity the detection method is appropriate. Detection was carried out separately for each channel and spikes were time stamped (according to UTC/GMT) to the nearest 0.1 s. Ictal periods were excluded from spike detection.

Automated spike detection was validated by comparing the algorithm output to expert annotation in randomly selected, 1 h data segments. Data segments were selected to evenly cover periods that were interictal, pre-ictal, and during night and day. The total spike count, true positive rate (TPR) and false positive rate (FPR) were evaluated. True positive rate was the number of marked spikes that were also detected by the algorithm, divided by the total marked spikes. False positive rate was the number of detected but unmarked spikes, divided by the total detected spikes.

There is no clear definition that can enable different experts to reliably identify interictal spikes in EEG. A recent study to validate a spike detection algorithm found that two expert detections showed only 41% agreement (Gaspard *et al.*, 2014). We designed our detection algorithm to err on the side of over-counting spikes, under the assumption that false positives would be evenly distributed, or at least would not strongly bias the distribution of true positives. The benefit of using a template-matching algorithm is that false positives are quantifiably 'spike-like' in morphology. Therefore, false detections may still reflect epileptic activity of interest (such as bursting).

Statistical analysis

Except where otherwise indicated, statistical analyses were carried out using MATLAB (version 7.10.0, R2013b, The Mathworks Inc., MA, USA). Spike data were analysed in terms of the rate over various time intervals, from minutes to days. Due to the automated detection algorithm, all extracted spike epochs were the same duration (300 ms).

A number of approaches were used to investigate the periodicity of spike rate and seizures. Colour-coded raster plots were used to display every seizure for each patient over the trial duration for easy visualization. Due to the density of spike occurrence, raster plots were not constructed for spikes. Circadian rhythms of spike and seizure rate were investigated graphically. Hourly rates were averaged over days, and then normalized to be between zero and the maximum rate. This provided an approximation to the probability distribution of spike and seizure occurrences conditioned on time of day. Sample autocorrelation (Box *et al.*, 1994) was calculated to establish the significance of repeating patterns observed in the data. Significant autocorrelation indicates that the level of predictability (using an autoregressive model) of spike or seizure rate was sufficiently unlikely to be observed by chance alone. For spike rate, autocorrelation lag times of 1 h to 24 h and 1 day to 30 days were used to enable infradian and ultradian rhythms to be investigated. The autocorrelation of the seizure rate with a 30-day lag was also calculated (due to the low average hourly seizure rate it was not possible to calculate autocorrelation over 24 h).

The relationship between interictal spikes and seizures was also explored using measures of cross-correlation. Spearman's rank correlation (Spearman, 1904) and Wilcoxon rank-sum (Wilcoxon, 1945) values were calculated between spike rate and seizure rate. These particular non-parametric correlation methods were used because the data for spike rates of many subjects departed from the normal distribution, demonstrated using the Shapiro-Wilk test (Shaphiro and Wilk, 1965).

A possible source of error in the data analysis was telemetry dropouts, where the electrical recording was temporarily interrupted. The majority of dropouts had durations of < 2 s and so did not limit our ability to record seizures, although the

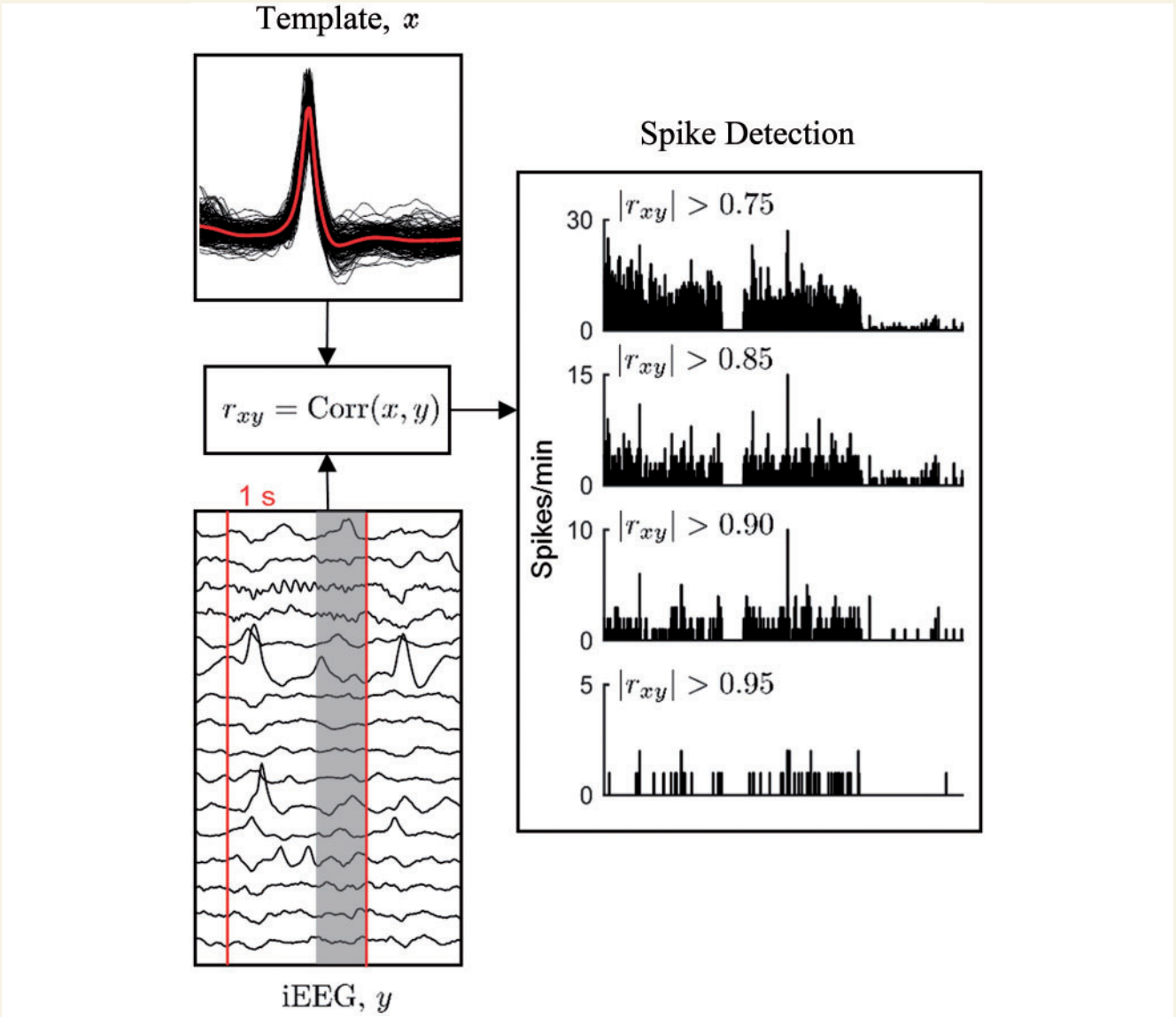

Figure 1 Example spike detection. A template is made by averaging similar spike patterns. A 1 s sliding window of 16-channel intracranial EEG (iEEG) is used to determine background amplitude. The final 300 ms (shaded region) is matched with the template. The absolute correlation coefficient between EEG and template, $|r_{xy}|$ is thresholded for spike detection. Spikes are rejected if amplitude is not within an acceptable range of the background. The right-hand panel shows example detection output (summed over all 16 channels) for different threshold values.

recorded spike rate may have been altered. To determine any relationship between the ability to record epileptic activity and the time spent in dropouts, we tested correlation between (time in dropout)/day and (time in seizure)/day. For seizures, statistical significance ($P < 0.05$) was found in the resulting correlation coefficients for three of the subjects. However, all of these demonstrated positive correlation, which is inconsistent with dropouts hindering seizure detection (where increased dropouts would result in reduced time in seizure), and was more likely to have been caused by coincidentally low rates of both seizures and dropouts. Therefore, it was unlikely that the dropouts affected the detection of seizures. For spike rates, correlation was tested between dropouts/hour and spikes/hour. Significant correlation ($P < 0.05$) was found between spike rate and dropout rate for all but two subjects. This is to be expected as clearly the more dropouts there are, the fewer spikes that will be detected, particularly for subjects with sustained spiking. However, in every case, the coefficient of correlation was low ($r < 0.1$), indicating that the observed spike rate was not overly affected by the dropout rate. In general, as results were obtained over very long-term averages (up to 2 years) and the majority of days in the trial contain no (or very few) telemetry dropouts, data were assumed to be sufficient to draw conclusions with low biases.

Table 1 Subject demographics and spike rate

Subject	Sex	Age	Seizure prediction sensitivity	Spike rate/min		No. of seizures		
				Mean	SD	Type 1	Type 2	Type 3
1	M	26	0.86	1.45	2.27	63	82	6
2	M	44	1.00	0.02	0.20	31	1	0
3	F	22	0.56	1.41	3.31	160	138	1272
4	M	61	0.71	7.58	10.85	17	5	0
5	F	20	E	0.08	0.57	6	3	2
6	M	62	NS	6.89	10.02	35	36	71
7	M	52	NS	0.56	1.91	93	243	260
8	M	48	0.63	1.11	1.86	210	233	3
9	F	51	0.18	4.22	7.09	135	22	592
10	F	50	0.54	1.39	2.49	258	146	688
11	F	53	0.56	0.94	1.65	177	288	14
12	M	43	NS	2.01	3.14	9	6	4558
13	M	50	0.57	15.79	12.03	140	335	514
14	F	49	1.00	4.12	6.72	10	1	0
15	M	36	0.71	42.16	15.74	53	12	10

Spike rate as spikes/min (SD = standard deviation). Prediction sensitivity refers to prediction of type 1 and type 2 seizures during the clinical trial. E = explanted; NS = initial phase performance not satisfactory to continue trial.

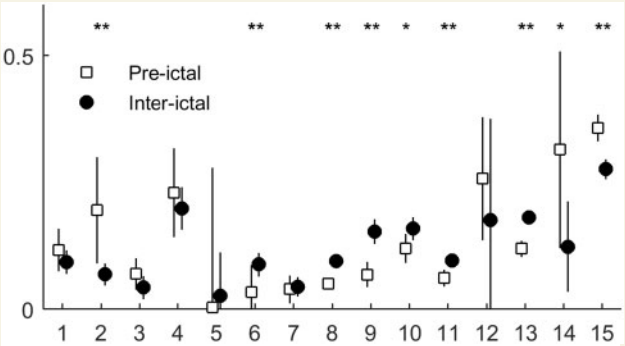

Figure 2 Comparison of average spike rate during interictal and pre-ictal periods. The hourly spike rate during pre-ictal windows was compared with the hourly spike rate during interictal windows. The pre-ictal window was defined as a 5 h seizure-free window preceding seizure. Significance was tested using the Wilcoxon rank sum test (*$P < 0.05$, **$P < 0.01$). Plot bars represent the median spike rate obtained over the entire pre-ictal (open squares) and interictal (filled circles) data. Error bars indicate the 95% confidence interval. For each subject, the spike rate was normalized to the range [0 1].

Results

Spike rate

The mean and standard deviations for spike rate (per min) for each subject are given in Table 1. Overall, the spike rate showed greater variance than mean across all subjects, which indicates that using a Poisson distribution to model spike count would not be appropriate. It would not be expected that a homogeneous Poisson distribution would describe the spike count well over an extended period of time, as there are most likely multiple, time-varying factors underlying spike generation. The average spike rate for most subjects was around one spike per minute, although there were extremes of low and high spike rate.

Figure 2 shows the median hourly spike rate obtained during pre-ictal periods compared to interictal periods. The pre-ictal period was defined as 1 h prior to a leading, clinical or clinically equivalent seizure, with 8 h used as a lead time. Interictal periods were defined as being at least 8 h before any seizure and more than 8 h after a seizure. Of the five subjects with the best seizure prediction performance in the original clinical trial (Subjects 1, 2, 4, 14, and 15) (Table 1), three showed significantly increased spike rate pre-ictally ($P < 0.05$, Wilcoxon rank-sum test) and the other two showed this trend, but not significantly. The remaining six subjects, who had significant ($P < 0.05$) changes in pre-ictal spike rate, all showed decreased spiking prior to seizure. See the online Supplementary material for test values and exact P-values.

Ultradian rhythms

Figure 3 shows patterns in seizure onset times relative to time of day over the duration of the recording period. Each seizure is displayed as a colour-coded (by seizure type, i.e. clinical, electroencephalographically identical, and subclinical) dot at the time of occurrence, with the horizontal axis extending for two consecutive days to display periodic variation. The grey shading in the panels indicates hours between 10 pm and 6 am. The consistency of seizure onset times at particular times of day is pronounced for some

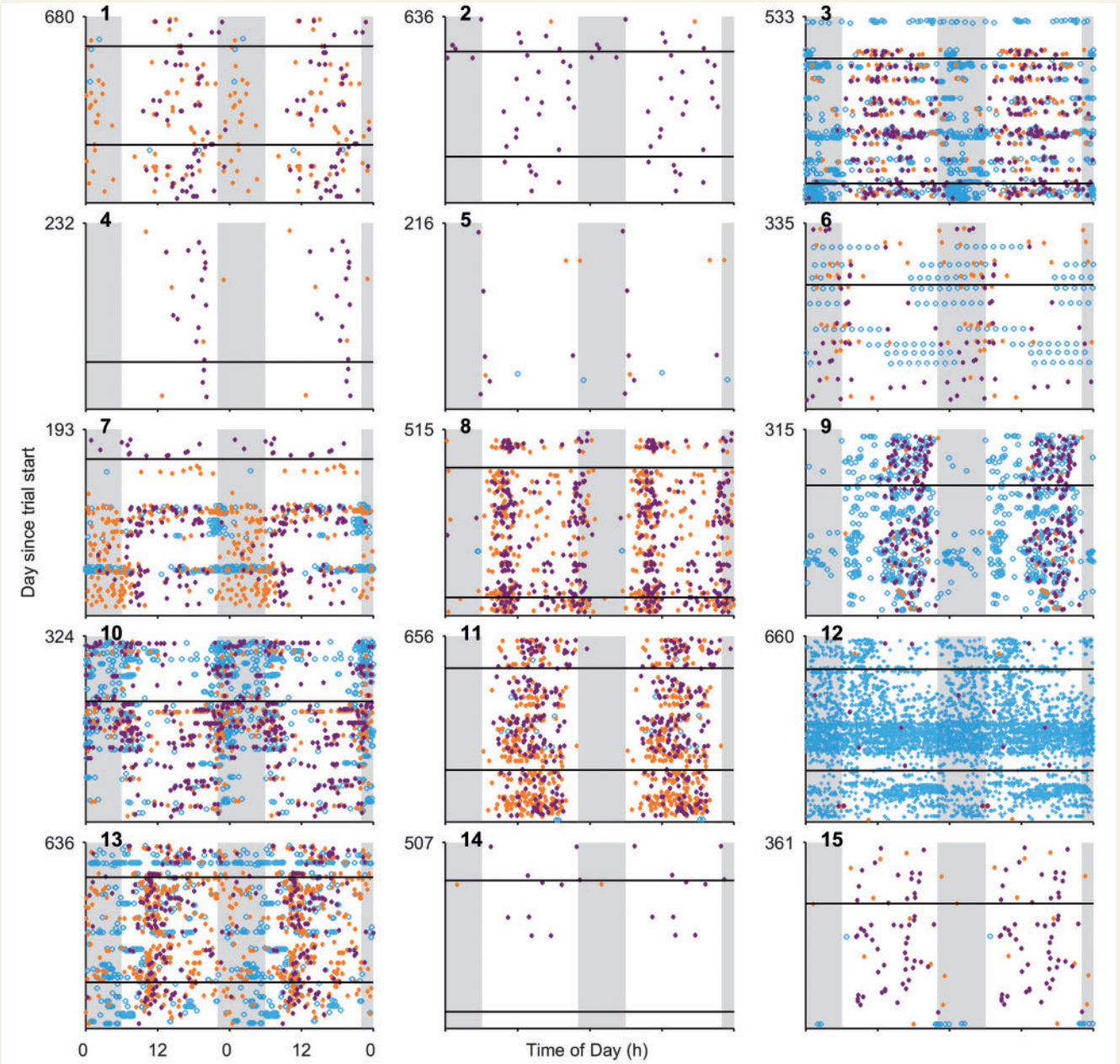

Figure 3 Seizure times by subject. Each subplot is a raster plot representation of the time of every seizure for a given subject. Plot colours indicate seizure type: purple, clinically recognized events; orange, electroencephalographically identical but unrecognized events; blue, electroencephalographically distinct subclinical events. The *x*-axis shows hour of day (AEST) for two consecutive days (i.e. the rows of the raster plot contain: row_1 = [day_1 day_2], row_2 = [day_2 day_3], ... row_{N-1} = [day_{N-1} day_N]). The *y*-axis represents *N*–1 consecutive days of the trial, where *N* is different for each subject (between 6 months and 2 years). Note that different subjects were enrolled from different start dates, and that horizontal lines locate 1 January. The grey shaded regions represent the hours between 10 pm and 6 am.

subjects even over many days to months. A relationship between time of day and seizure onset is demonstrated by the clear vertical arrangement of data in Fig. 3 (Subjects 4, 8, 9, and 11 are good examples of this).

There is evidence of a persistent relationship between seizure onset times and the day-night cycle. It is likely that this pattern is related to sleep. However, times of the sleep-wake cycle were not recorded during the trial. Nevertheless, Subject 8 demonstrates two times of day where seizures are clustered (vertical bands in the plot), and these occur at times where transitions to or from sleep are likely to occur. Subject 4 is also noteworthy in the same regard; although few seizures were recorded for this subject, they appear to be well aligned within a time span between 6 pm to 9 pm. It is possible that these seizures are also related to the transition to sleep.

There are other patterns evident in Fig. 3 although these are patient-specific and as such, are difficult to characterize. However, these patterns hint at a level of predictability in the temporal organization of seizures for certain subjects. For instance, Subject 9 shows a consistent pattern, where a cluster of subclinical events precede clinically-relevant seizures. This is also the case for Subjects 3, 7, and 13, although Subject 7 has reported clinical events preceded by clusters of unreported but electroencephalographically identical events. Subject 6 shows a series of subclinical events before clinically-relevant seizures and, interestingly this pattern does not manifest early on in the trial (towards the bottom of the panel). Subjects 7, 10 and 12 also show signs of long-term variability in patterns of seizure occurrence, indicated by inconsistency in the vertical direction. Note that Subject 12 is unusual in that thousands of subclinical events were detected. It is also worth noting that subjects with high rates of seizure do not necessarily demonstrate the highest rate of interictal spiking. For instance, Subject 15 does not show many seizure events but recorded almost continuous spiking (averaging 42 spikes/min, see Table 1).

Figure 4 shows an empirical approximation of the probability density for spikes and seizures (only clinical, type 1 or clinically equivalent, type 2 seizures were included) conditioned on time of day. The peaked shape of these distributions provides evidence that both spikes and seizures occur with circadian rhythm, as spike or seizure likelihood is enhanced at particular times of day. Note that the plot values are repeated on the x-axis in order to visualize peaks that cross midnight. Some subjects do not demonstrate such obvious cyclic rhythms, which may be due to insufficient data, particularly with regard to the number of recorded seizures. The phase of circadian oscillation is shifted for different subjects, with evidence of peaks during the day and night, although diurnal peaks are more common. Typically, the peak times for both spike and seizure peaks are well aligned or at least are not antiphasic (Subjects 2, 6 and 11 are the possible exceptions here). This synchrony suggests that, on average subjects show a higher probability of epileptic events at particular times of day. Note that although subclinical (type 3) seizures were not included in the analysis in Fig. 4, spike detection was not performed during subclinical seizures; therefore the distribution of subclinical seizures may alter the distribution of spikes. It can be seen in Fig. 3 that only Subjects 3, 9 and 10 demonstrate temporally aligned subclinical seizures.

Figure 5 shows the autocorrelation function for the hourly spike rate with a 24 h lag time. The autocorrelation can be interpreted as the level of predictability inherent in spiking or seizure rate. For instance, increased correlation at a lag of 24 h indicates that to predict current spike rate, it is useful to know the spike rate from 24 h ago. This was found to be the case for most subjects, indicating a repeating pattern with a 24-h period. Subjects that did not demonstrate a clear cycle (Subjects 7, 10 and 12) showed consistently high autocorrelation over the entire 24 h cycle, indicating that the history of the whole preceding day was relevant to predicting the current spike rate.

Infradian rhythms

Cyclic behaviour over longer time scales was also investigated. Figure 6A shows the autocorrelation function for daily spike rate over 0 to 30 days (the age and sex of each subject is presented in Table 1). There is less evidence of cyclic behaviour compared with the circadian autocorrelation plots. However, Subject 6 (male) showed evidence of a monthly cycle in spike rate (clear increase at a lag of 30 days). There are signs of periodicity over less than a month, for instance Subjects 9, 10, 11 and 14 (all female) show peaks at lag times approximately between 1 to 3 weeks. Subject 12 also shows a high autocorrelation over the entire month, indicating their daily spike rate was consistent over a long term.

Figure 6B shows the autocorrelation of seizures per day over a 30-day period. Although there are no obvious 30-day cycles, there is evidence of shorter term periods in subjects with significant correlations at lags between 1 week to 1 month (Subjects 3, 6, 8 and 9–12). These also showed significant correlation in spike rate over a 30-day period, with the exception of Subjects 3 and 8. It should be noted that these two subjects have similarly located peaks in the spike rate autocorrelation function (Fig. 6A), which were not considered significant. Subjects 14 and 15 showed significant correlation in spike rate but not for seizure rate. Subject 12 showed high correlation over the entire month interval, most likely due to a very high rate of subclinical seizures.

Discussion

This study represents the first long-term, ambulatory analysis of the relationship between interictal spikes and seizures. The results provide fundamental insights into the mechanisms of epilepsy and new avenues of seizure control, which we discuss in more detail below. Half (9 of 15) of the subjects showed a significant change in spike rate prior to seizures, indicating that spikes are not wholly independent of seizures. Of these subjects, spike rate typically decreased pre-ictally (in 6 of 9 subjects). However, it is interesting to note that the subjects whose spike rate increased also had the best seizure prediction results in the clinical trial (Cook *et al.*, 2013). The prediction algorithm used measures of signal energy in various frequency bands; therefore we can speculate that increased spiking activity may have contributed to seizure prediction for these subjects. However, the relationship between epileptic spikes and seizures was subject-specific. It is not clear that spikes directly promote or inhibit seizures, however, we hypothesize that spikes and seizures share one or more common regulatory mechanisms, as evidenced by their similar circadian patterns.

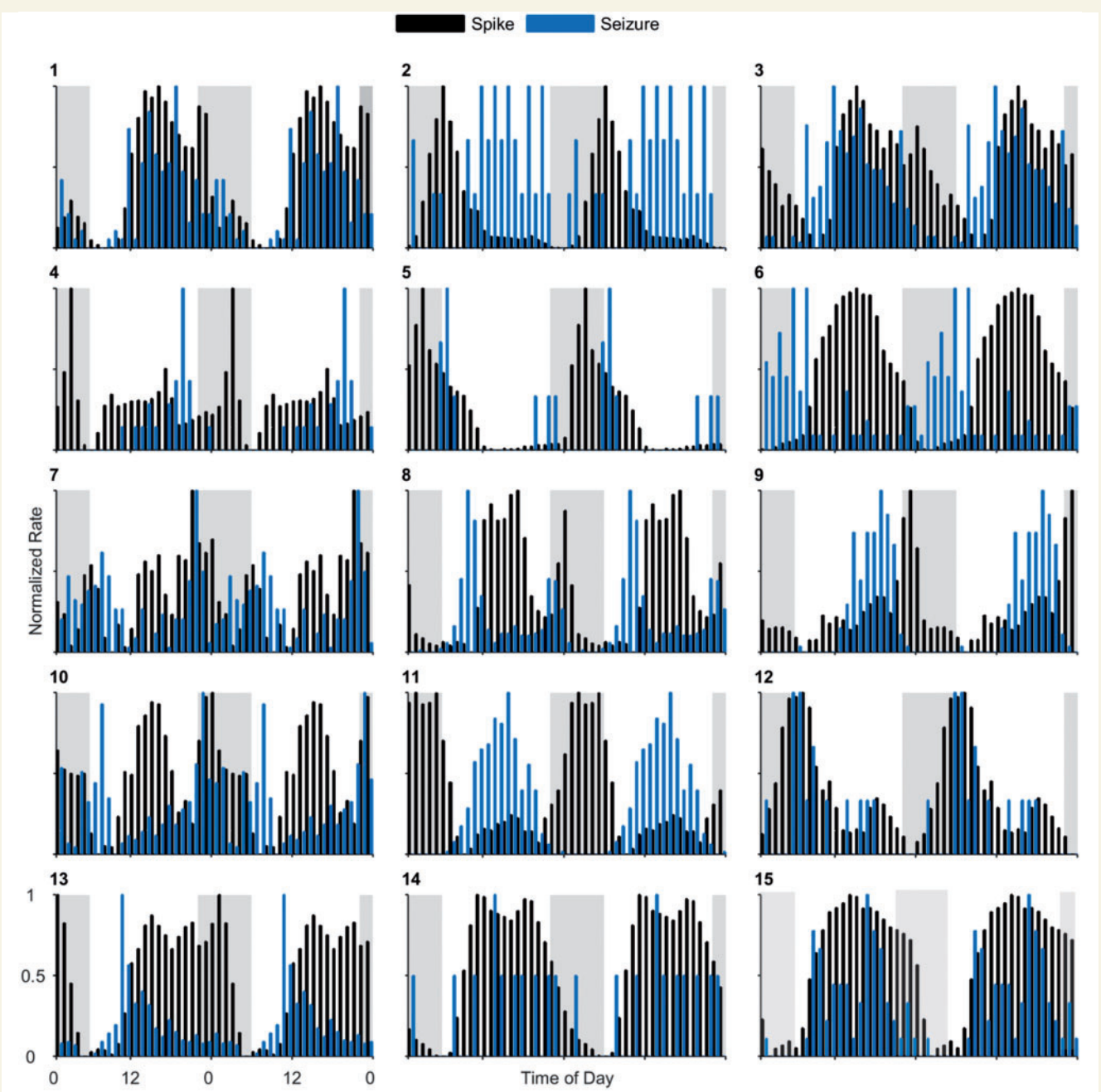

Figure 4 Circadian rhythm in spike and seizure. Each subplot is a bar graph representing the average number of spikes (black) or seizures (blue) per hour. Only clinical (type 1) and electroencephalographically identical (type 2) seizures were included. The average hourly rates were then individually normalized to the range [0 1]. The resulting values are repeated on the *x*-axis in order to visualize circadian patterns. The grey shaded regions represent the hours between 10 pm and 6 am.

Periodicity of spikes and seizures

It has long been demonstrated that circadian cycles influence seizure occurrences in a subject-specific manner and, as we have shown here, there was a consistent relationship between seizures and time of day (Fig. 3). Epileptic spikes were shown to follow a similar (and also subject-specific) circadian variation and, compellingly, the peaks in the distributions of spike times and seizure times within subjects were typically located at similar times (in all but three subjects). We propose that the similar peak times for spike rate and seizure rate indicate a common regulatory factor influencing the generative mechanisms of both. A possible mechanism is the effect of sleep on spike and seizure generation. Although sleep data were not recorded for this study, it is known that sleep cycles are related to seizure onset times (Patry, 1931; Baldy-Moulinier, 1986; Crespel *et al.*, 2000). There is also previous evidence that alertness or

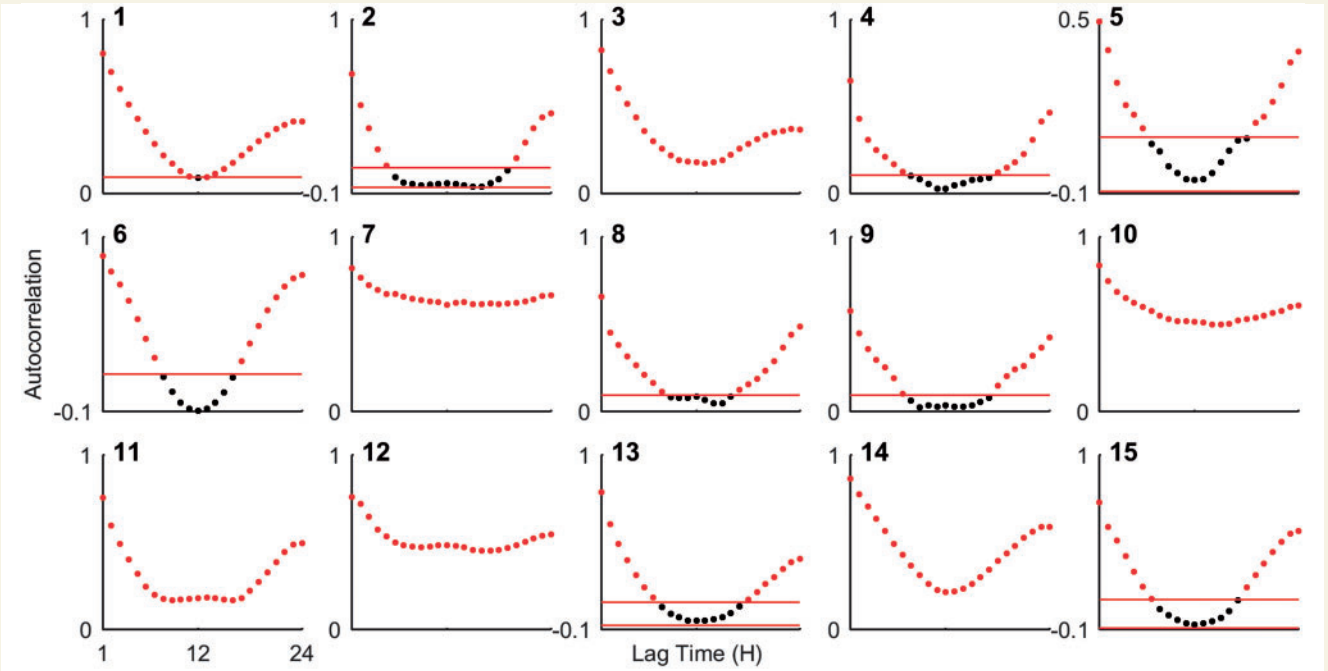

Figure 5 Autocorrelation function for spike rate over 0–24 h. The autocorrelation function between successively recorded values of spikes/h is plotted from a lag of 0 to 24 h. Note that the autocorrelation function is normalized to the value at 0 h. The red lines indicate the 99% confidence interval (with Bonferroni correction for multiple comparisons over the *N* days in the trial) that correlation values are significant. Points considered significant are coloured in red. Confidence limits are omitted where all points obtained significance.

sleep stage influences spike generation. For instance, it was noted that spike rates may be enhanced during periods of drowsiness (Ward, 1959) and also that spikes undergo short-term cycles correlated with sleep stages (Ferrillo *et al.*, 2000). Further investigation is warranted into possible causes of subject variability in the phase (peak location) of spike rate and seizures. It is possible that different peak locations are related to different network involvement in seizure onset, as disruptions in certain circadian regulatory pathways have been hypothesized to lead to a pro-ictal state (Quigg, 2000; Stanley *et al.*, 2013).

Some subjects showed evidence of longer cycles (weeks to months) in spike rate and seizure rate. The appearance of monthly periodicity in seizure times related to menstrual cycles in females is fairly well established (Backstrom, 1976; Taubøll *et al.*, 1991). However, there is no proven explanation as to why males can show evidence of catamenial cycles of epileptic activity, although there are a number of studies demonstrating their existence (Bercel, 1964; Newmark and Penry, 1980; Bauer and Burr, 2001). In this study the subject with the strongest evidence of monthly periodicity in spike rate was male (Fig. 6A, Subject 6), although more female subjects showed evidence of week to month long periodic cycles. The autocorrelation functions for seizure rate and spike rate (Fig. 6A and B) generally showed peaks at similar locations, which is further evidence of a common regulatory mechanism influencing spike and seizure generation.

Identifying candidate mechanisms that cause both ultradian and infradian rhythms is worthy of further study, as this may shed light on mechanisms of seizure generation. It is extremely difficult to study most biophysiological factors in conjunction with seizure rhythms due to the paucity of data. However, interictal spikes are more frequent, can be recorded non-invasively, and, as we have seen, demonstrate predictable daily fluctuations. Identifying the cause of periodicity in spikes may provide testable hypotheses as to the cause of periodicity in seizures. A better understanding of the slow acting regulatory mechanisms underpinning epileptic activity will improve timing of medication and may even suggest new treatment strategies.

Relationship between spikes and seizures

There is no firm consensus on the link between interictal spikes and seizures. It is possible that spikes are caused by different cellular and network mechanisms to seizures (Gotman, 1991; De Curtis and Avanzini, 2001). Another proposal is that interictal spikes and seizures are not independent events, but the relationship may vary with other factors such as onset location or brain state (Laetitia *et al.*, 2012). Here we showed spike rate was significantly changed prior to seizures in over half of the subjects. Increasing spike rate was linked to improved seizure prediction, although the increase could equally be attributed to spikes

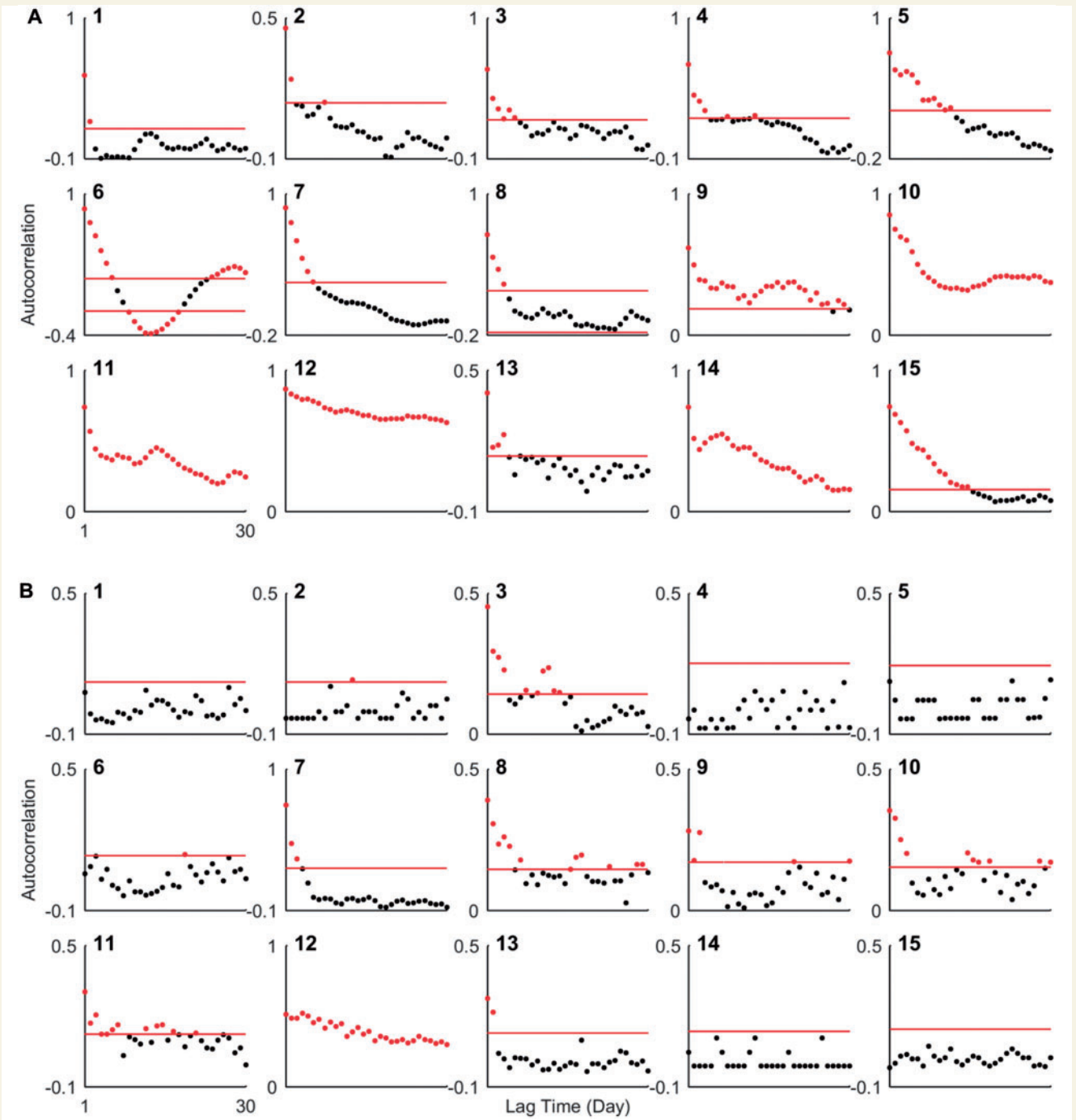

Figure 6 Autocorrelation function for 0–30 days. (**A**) The autocorrelation function between successively recorded values of spikes/day is plotted from a lag of 0 to 30 days. (**B**) The autocorrelation function between successively recorded values of seizure/day is plotted from a lag of 0 to 30 days. Note that the autocorrelation function is normalized to the value at 0 days. The red lines indicate the 99% confidence interval (with Bonferroni correction for multiple comparisons over the number of months in the trial) that correlation values are significant. Points considered significant are coloured in red. Confidence limits are omitted where all points obtained significance.

promoting seizures, or a secondary regulatory mechanism facilitating epileptic events. Other subjects showed decreased spiking before seizures, which adds weight to the existing evidence that spike rate can also decrease prior to ictogenesis (Engel and Ackermann, 1980; De Curtis and Avanzini, 2001; Avoli *et al.*, 2006). However, this does not mean that decreased spiking is causally linked to having a seizure (i.e. it does not show that spikes protect against seizures). On the contrary, for the majority of subjects, high likelihood of spikes was associated with high likelihood of seizures (shown in Fig. 4 where peaks occurred at similar time of day). This similarity in the

shape of the probability distributions suggests a common regulatory factor between spikes and seizures. As spikes involve a massive, synchronous neuronal discharge, it is possible that their generation could be regulated by similar global or local changes in excitability or enhanced synchrony that also promote seizures.

The significance of a subject-specific pre-ictal decrease in spike rate is controversial (De Curtis and Avanzini, 2001; Avoli *et al.*, 2006). It is possible epileptic spikes provide an active protective benefit against seizures. Therefore, a decrease in spiking prior to seizures can be interpreted as a failure of a regulatory mechanism. If this is the case, preventative action of the spikes could be augmented by treatment strategies. For instance, results have shown drugs that depress interictal spiking may have the opposite effect on ictal activity (Watts and Jefferys, 1993; Barbarosie and Avoli, 1997) and electrical stimulation that promotes spike-like responses can suppress seizures (Swartzwelder *et al.*, 1987). Recordings from animal kindling models of epilepsy show that interictal spiking decreases in parallel with decreased seizure thresholds, and inducing spikes can increase the seizure threshold (McNamara *et al.*, 1980). However, this does not constitute unambiguous evidence that spikes prevent seizures, as there may be a secondary mechanism involved. We have hypothesized that similar regulatory factors lead to increased probability of both spikes and seizures. This brain state (where both events are more likely) may be necessary and sufficient for spikes, but insufficient for seizures. It is even possible that an attempt to suppress spikes may lead to greater risk of seizures. We raise these speculative hypotheses to highlight the complexity involved in interpreting the available evidence. It is necessary to develop further experiments capable of distinguishing between the possible causal or indirect relationships.

Implications for prediction

The very clear evidence that seizures occur reliably, for some subjects almost exclusively, at particular times of day is highly relevant to prediction. These patterns were relatively stationary and, for most patients, were evident early in the clinical trial (Fig. 3). The consistency in patterns of seizure and spike activity over long periods (up to 30 days) was demonstrated through autocorrelation plots, indicating that knowledge of past spike or seizure rate may be relevant to predicting current events. Therefore, information about the history of seizure timing and spike or seizure rate could be used to weight the outcome of seizure prediction algorithms, potentially improving performance either through increased accuracy or reduced false positives.

In this study we noted a link between good seizure prediction performance and increased pre-ictal spiking. It is possible that for these subjects increased spike rate is an indication of imminent seizure, thus aiding prediction. We also noted that the likelihood for spikes and seizures showed similar patterns (Fig. 4). Based on this observation, it is possible that prediction algorithms may appear to be incorrectly predicting seizures while in fact correctly identifying smaller epileptic events. Therefore, a method of distinguishing between the two is necessary. Model-based prediction that is capable of uniting a range of epileptiform behaviours is a technique that may assist with this (Deco *et al.*, 2008; Freestone *et al.*, 2014; Wang *et al.*, 2014). Training prediction algorithms to recognize other epileptic events may also be a useful strategy to recognize times of heightened risk of seizure, and possibly guide medication delivery.

Regardless of whether the pre-ictal decrease in spike rate is a secondary symptom of the brain approaching seizure, or a causal factor, spikes could be used to aid prediction. Although for those subjects who show decreased spiking, prediction strategies should focus on extracting different information from interictal spikes (rather than a simple rate). For instance, if spike rate is modulated by common regulatory factors as seizures then spikes can be used as biomarkers of cortical excitability. It has been shown that the cortical response to stochastic inputs can provide an early warning sign of an impending seizure (Negahbani *et al.*, 2015). Similarly, features of spikes, such as spatiotemporal distribution or morphology, could be used to measure the resilience of the underlying system. Previously, electrically evoked potentials have been used to quantify the cortical response in order to estimate seizure likelihood (Kalitzin *et al.*, 2005; Freestone *et al.*, 2011) and applying this approach with interictal spikes warrants further investigation.

It should be noted that the subjects in this study were not chosen specifically to investigate the relationship between seizure and other interictal spikes. Therefore, it is difficult to relate the findings of this study to more general properties of seizures and epilepsy due to the potentially confounding influences of multiple medications and, in some cases, previous surgeries. However, as epilepsy is a highly subject-specific disease with few demonstrably general properties, valuable insight can still be gained from particular cases and these may also suggest pertinent directions for future research.

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Supplementary material

Supplementary material is available at *Brain* online.

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