Intraindividual Characterization of the Sleep Spindle Variability in Healthy Subjects

J. Gomez-Pilar, G. Northoff, F. Vaquerizo-Villar, J. Poza, *Senior Member, IEEE*, G. C. Gutiérrez-Tobal, *Member, IEEE*, and. R. Hornero, *Senior Member, IEEE*

***Abstract*— Spatial and frequency characterization of sleep spindles have been extensively addressed using M/EEG or fMRI recordings. However, its intraindividual variability across time has not been addressed. Here we propose to assess the intraindividual variability of sleep spindles in a time-resolved way by means of a trial-to-trial-variability (TTV) measure. For that purpose, the EEG of 26 healthy subjects were recorded overnight. After an exhaustive preprocessing pipeline to remove artifacts, spindles were automatically detected using a complex demodulation-based method. Then, the Wavelet Scalogram was estimated to validate it. Spindle TTV of each participant was also computed for all the conventional EEG frequency bands. Root mean square (RMS) of each TTV signal was calculated as a measure of the total variability of each spindle. Results showed significant differences in the variability between frequencies. Specifically, RMS in the beta-1 frequency band showed higher values as compared to all the other frequency bands ($p<0.001$). TTV curves showed a dichotomic trend, with lower frequencies showing an increase in the variability before the spindle onset, and higher frequencies showing such increase after the onset. The dependence of the spindle variability with the frequency could be explained by the influence of the multiple cortical generators involved.**

***Clinical Relevance*— Sleep spindles are similarly affected in different cognitive-related disorders, which supports the relevance of assessing abnormal sleep patterns as a possible cause for such cognitive deficits.**

I. INTRODUCTION

Sleep spindles are characteristic electroencephalographic (EEG) bursts around the sigma frequency band (11-17 Hz), which usually last between 0.5 and 2 s [1]. Spindles are more frequent in the second phase of non-rapid eye movement sleep (N2), other phases of sleep can contain spindles thought [1]. During the last years, several attempts to characterize their intracortical neural pathways have been made [2], [3]. However, the role of the activation of brain cortical generators and their dependency with frequency, remain relatively obscure. Actually, whereas most of the spindles appear synchronized and widely distributed on the cortex, local spindles exist too [4], suggesting different mechanisms and different functions.

It is well-known that spindles are not isolated events. Indeed, a hierarchical nesting of slow oscillations (SO), spindles, and ripples is frequent during sleep [5]. Particularly, spindles are modulated by the up-state of the SO (0.75 Hz), which in turn mediate in the generation of high frequency bursts (around 100 Hz in human hippocampus), *i.e.*, ripples [5], [6]. It is thought that the hierarchical combination of these three signals is responsible for sending information units to distributed neocortical sites for long-term storage [5].

Supporting these findings, diverse studies have associated basic sleep spindles features with the intelligence quotients (IQ) [7], as well as several cognitive traits [8], [9]. Interestingly, there are studies that have provided evidence on the relationship between reduced activity or disrupted spatiotemporal evolution of sleep spindles and certain neurological, psychiatric, and respiratory diseases, such as Alzheimer's disease [10], schizophrenia [11] or sleep apnea [12]. In this context, sleep spindle characterization is becoming a crucial tool to elucidate the role of sleep in brain plasticity [13], memory [2], [3], [14], and intelligence [7].

Although the above mentioned hierarchical nesting frequency results in a complex electrophysiological signal (SO-spindles-ripples), most of these studies only involved basic features from sleep spindles to establish associations between the electrophysiological signals and cognitive traits. Among these basic features, the spindle density (number of spindles per minute), total number of spindles, spindle duration, and spindle amplitude stand out [5]. Much less frequently, spectral measures have been also used to characterize spindles by means of its frequency, power or coherence [15]. However, all these studies assessed spindle features using grand-average measures across subjects, leaving aside the intraindividual spindle variability throughout the sleep time.

Against the general trend, negative results have also been achieved. One example is the lack of correlation between spindle features and specific cognitive traits (correlated in other studies) [7]. At least in part, it may be due to the fact that

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J. G-P. (corresponding author e-mail: javier.gomez@gib.tel.uva.es), F. V-V., J. P., G.C. G-T., and. R. H., are with the Biomedical Engineering Group from the University of Valladolid and CIBER-BBN (ISCIII), Spain

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certain characteristics of the spindles have been only considered in terms of mean. However, a combination of different types of spindles (*i.e.*, spindle variability) could be the basis for the underlying mechanisms responsible for appropriate long-term storage in neocortical sites. Supporting this assertion, opposite correlation has been found between "fast" and "slow" spindles and IQ measures (*e.g.*, [16]). These studies provide previous evidence on the importance of spindles variability and their dependence on the frequency for correct cognitive development. We here hypothesized that, despite the relatively narrow frequency band of the spindles, the spindle variability is frequency-dependent, showing different behavior for each conventional frequency band of the EEG. In other words, the waves in which spindles are embedded not only modulate the spindle amplitude or frequency, but also its variability throughout the night.

On the basis of the above hypothesis, this study is aimed at analyzing the spindle variability in the conventional EEG frequency bands in order to assess the spindle dynamics and its dependence with the frequency. For this purpose, after an automatic spindle detection, trial to trial variability (TTV, an index of the instability degree) was computed for all subjects in the EEG frequency bands.

II. MATERIALS AND METHODS

A. EEG recordings and preprocessing

Twenty-six healthy adults (age =26.00 ± 6.69 years, 18 women) free from sleep disorders were recruited in this study. Participants underwent overnight polysomnography (PSG) using the Embla Titanium PSG system (Natus, San Carlos, CA). EEG, electrooculographic (EOG), and electromyographic (EMG) signals were acquired with impedances < 5 kΩ, at a sampling rate of 500 Hz, referenced to FPz. EEG was acquired using 11 gold-plated electrodes placed according to the conventional 10-20 system.

The EEG signals were re-referenced offline to the average activity of the mastoid derivations for sleep stage scoring. Sleep stages (wake before sleep, N1, N2, N3, REM), as well as noisy segments, were marked using RemLogic analysis software (Natus) in epochs of 30 seconds, as recommended by the American Academy of Sleep Medicine [1]. After removing noisy epochs, signals were filtered using a band-pass finite impulse response (FIR) filter between 1 and 70 Hz using a Hamming window. Finally, an adaptative epoch rejection protocol using a statistical-based thresholding method was applied: (i) the mean and standard deviation of each channel was computed, (ii) epochs that exceeded mean ± 4 × standard deviation in at least two channels were discarded [17]. After resampling EEG data to 250 Hz, automatic spindle detection was carried out in the cortical midline electrodes (Fz, Cz and Pz) using a previously validated method [18]. Spindles were then segmented from 1 second before the spindle onset to 2 seconds after it, defining each temporal window as a single trial, *i.e.*, $x[n]$ with n representing samples from -250 to 500. All the preprocessing was applied using in-house MATLAB (The MathWorks, 2017b) scripts and the EEGLAB toolbox [19].

B. EEG processing: Wavelet Scalogram (WS)

Although the automatic spindle detection method has already been widely validated in previous studies [18], this dataset lacked sleep-related events labeled by an expert. For this reason, we decided to control for the right functioning of the method by means of a thorough time-frequency analysis.

The non-stationarity of the EEG recordings must be considered for such time-frequency analysis. When transient states of EEG are analyzed, as the case of spindles, non-stationarity becomes even more critical. This forces to use alternative methods to the classical Fourier analyses. In this study, Continuous Wavelet Transform (CWT) was used for time-frequency analysis. Specifically, the complex Morlet wavelet was chosen as "mother wavelet" to provide a biologically plausible fit to the EEG signals [20]. Bandwidth and wavelet center from the complex Morlet, as well as the dilatation factor for the CWT, were set as in [20] in order to obtain a balance between time and frequency resolutions.

The Wavelet Scalogram (WS) was subsequently computed from the CWT of each trial, channel and subject. WS provides a summary of the energy distribution (time and frequency) for each spindle detected. We here normalized the WS so that the summation across frequency was 1. Thus, it can be interpreted as a probability density function:

$$WS_n(k,s) = \frac{\|CWT(k,s)\|^2}{\sum_s \|CWT(k,s)\|^2}, \quad (1)$$

where k represents the translation factor and s the dilatation factor.

C. EEG processing: trial-to-trial variability (TTV)

With the aim of assessing the spindle variability across trials, TTV was computed for each subject. TTV index was defined as the normalized standard deviation (SD) in a time-resolved way. The normalization was conducted with respect to the SD at the spindle onset by a successive subtraction and division:

$$TTV[n] = \frac{std(x[n]) - std(x[0])}{std(x[0])}, \quad (2)$$

where $std(\cdot)$ represents the SD function and $x[0]$ is the EEG signal at the stimulus onset.

Normalized TTV index was then computed for the EEG conventional bands: delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta-1 (13-19 Hz), beta-2 (19-30 Hz) and gamma (30-70 Hz).

D. Statistical analysis

Root mean square (RMS) of the TTV index was computed as a statistic of the variability degree in each frequency band and subject. Then, after confirming that the parametric assumptions did not meet, Friedman test was used to assess the effect of the frequency on the RMS of the TTV. Finally, Wilcoxon signed-rank test was used for pairwise comparisons.

III. RESULTS

A. Sleep spindles statistics

Statistics about the total number of spindles automatically detected in the three selected channels, as well as their duration (mean and SD), are summarized in Table 1. Considering all participants together, more than 100,000 spindles were detected in Fz, Cz and Pz, with longer durations and higher recurrence on the parietal lobe (see Table 1).

TABLE I. SPINDLE STATISTICS, MEAN (SD)

	Electrode			
	Fz + Cz + Pz	**Fz**	**Cz**	**Pz**
# spindles per subject	4425.9 (720.1)	1398.2 (244.8)	1476.2 (250.3)	1551.4 (240.0)
spindle duration (seconds)	0.9 (0.3)	0.8 (0.3)	0.9 (0.3)	0.9 (0.3)

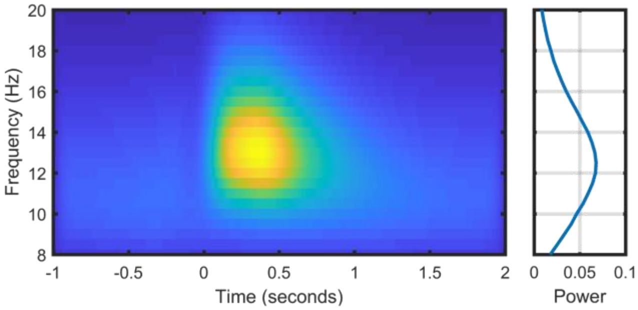

Figure 1. Averaged Wavelet Scalogram. The peak in power around 13 Hz after the spindle onset proves a suitable spindle detection by the algorithm. The colormap also reveals that most spindles have an average spindle duration shorter than 1 second.

In order to validate the automatic spindle detection procedure, WS was calculated for all the trials. Figure 1 shows the grand-averaged scalogram across trials, channels and subjects, along with the spectral power in such windows of 3 seconds (right panel). A peak of power was observed around 13 Hz in the period between the spindle onset and 0.5 seconds. The figure reveals that most of the spindles detected are shorter than 1 second, which is coherent with the average duration showed in Table 1 (0.9 s).

B. *Sleep spindles variability*

Once assessed the correct spindle detection, the normalized TTV index with respect to the spindle onset was calculated. Figure 2 shows the averaged spindle variability for each EEG frequency band. Depending on the band, a different trend of the TTV can be observed, which reveals a frequency-dependent nature of the spindle variability. Lower frequencies (delta and theta) showed a slight increase on the spindle variability before the spindle onset. By contrast, and higher increased variability was shown in alpha and beta-1, with beta-2 and gamma showing slight increase.

RMS was calculated for each participant to assess the variability degree of each band. Figure 3 shows the distribution of RMS for all the frequency bands. Friedman test revealed a statistically significant effect of the frequency band in the RMS distribution ($p<0.001$, $\chi^2=59.67$). Interestingly, beta-1 showed the higher variability degree, with statistically significant differences when compared with the remaining frequency bands ($p<0.001$, Wilcoxon signed-rank test). Similarly, alpha showed statistical significant differences with delta ($p=0.003$, $Z=2.94$) and theta ($p=0.003$, $Z=2.94$), but not beta-2 or gamma ($p>0.05$).

IV. DISCUSSION

Using overnight EEG, we investigated the dependence of the spindle variability on the frequency through the assessment of differences in the TTV for the conventional EEG frequency bands. RMS was used to quantify the degree of spindle variability for each participant and band.

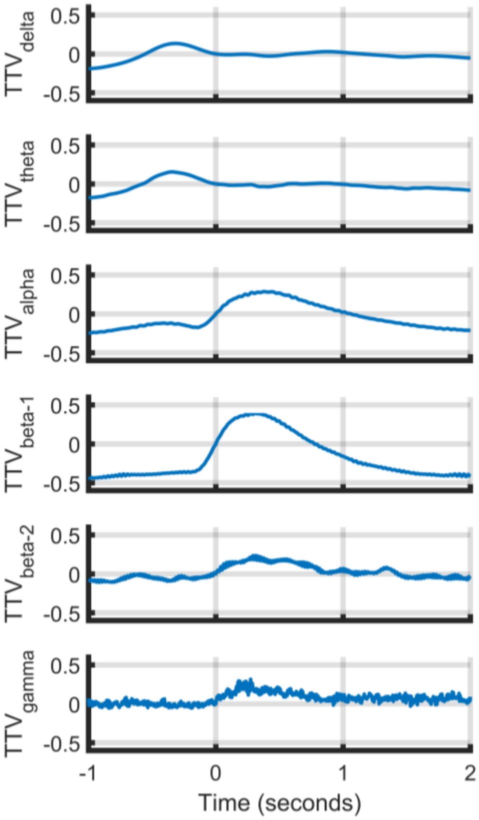

Figure 2. Spindle variability for each EEG frequency band by means of the normalized TTV index. Lower frequencies bands (delta and theta) show an increase on variability before the spindle onset. On the contrary, increased variability is shown in intermediate and higher frequencies (mainly in beta-1).

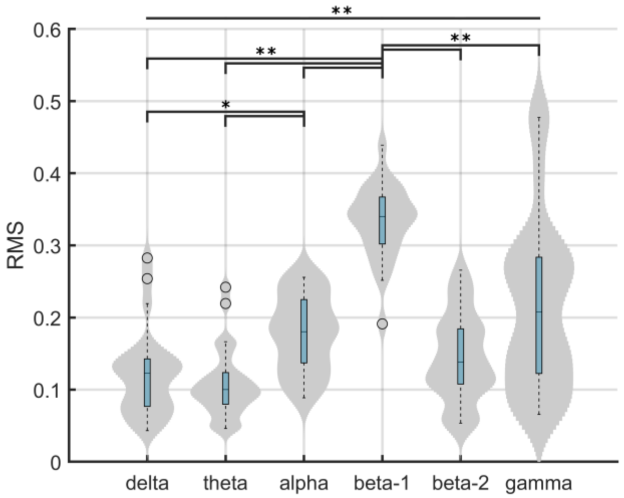

Figure 3. Root mean square of the TTV for each frequency band. A statistically significant effect of the frequency band was shown (** p<0.001, Friedman test), with higher values in the beta-1 frequency band.

TTV curves showed different shapes depending on the frequency band, with an increase in variability in the lower frequencies before the spindle onset, followed by an increase in the higher ones after it. These findings can be linked to the frequency nesting produced during spindle generation. Previous studies showed the relevance of the SO in bundling spindles [5], [14]. Concretely, it is though that the depolarizing effect secondary to the SO up-state facilitates the spindle generation [5]. This increase on variability previous to the spindle burst should be more evident on the lower frequency bands, which is exactly what Figure 2 shows. On the other hand, spindles produce grouped and embedded ripples in the spindles themselves [5]. Therefore, the high frequencies associated with ripples should appear after the spindle generation. This could be observed in higher frequencies as an increase on the variability of the EEG signal after the spindle onset, which again is showed in Figure 2. For both lower and higher frequencies, the observed EEG variability across trials time-locked with the spindle onset is linked to the probability of occurrence of SO and ripples, since spindles could appear alone, without being hierarchically nested [5].

A different explanation should be provided for intermediate frequencies as alpha and beta-1. As mentioned, sigma frequency comprises high alpha and low beta-1. During the spindle burst, an increase on the variance of the ongoing EEG signal is produced. This increase in signal energy is related to the peak observed on the WS (see Figure 1). However, it is important to note that an increase on the signal variance or energy does not involve an increase on the signal variability across trials, which is what Figure 2 shows by the TTV. To provide a possible explanation of the TTV increase, we rely on the additivity concept of the EEG. Previous studies proposed the linear superposition model as the basis in the EEG rest-task interaction (*e.g.*, [21], [22]). They suggest that the neural activity during an event evoked externally (exogenous activity) or internally (endogenous activity) overlaps in an additive and linear way with the ongoing activity [21], [22]. According to the Law of Total Variance, a direct consequence of this model is that the variance during the event (a spindle in this case) should always be increased. This model seems to be successful between 0 and 1 second approximately, where the variance of the SO, the spindles, and the ripples would be summed up in a linear manner. However, this model would be valid only for the intermediate and higher frequency bands. An alternative model has been also proposed; it is based on the nonadditive interaction between ongoing and event-related activity, which shows that the additive model is not valid under different conditions in fMRI [23] or electrophysiological recordings [24]. Our results extend these findings to suggest that, as for the spindle variability, the correct model could depend on the frequency.

V. CONCLUSION

The great dependence between spindle variability and frequency showed in this study is probably linked to the hierarchical nested signals that involve the spindle bursts. It emphasizes the relevance of multiple cortical regions on spindle generation. To reinforce this hypothesis, future studies should assess whether particular patterns of sleep spindles are related to cognitive functions depending on the cortical source involved in its generation.

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