A new quantitative automatic method for the measurement of non-rapid eye movement sleep electroencephalographic amplitude variability

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SUMMARY

The aim of this study was to arrange an automatic quantitative measure of the electroencephalographic (EEG) signal amplitude variability during non-rapid eye movement (NREM) sleep, correlated with the visually extracted cyclic alternating pattern (CAP) parameters. Ninety-eight polysomnographic EEG recordings of normal controls were used. A new algorithm based on the analysis of the EEG amplitude variability during NREM sleep was designed and applied to all recordings, which were also scored visually for CAP. All measurements obtained with the new algorithm correlated positively with corresponding CAP parameters. In particular, total CAP time correlated with total NREM variability time ($r = 0.596$; $P < 1E\text{-}07$), light sleep CAP time with light sleep variability time ($r = 0.597$; $P < 1E\text{-}07$) and slow wave sleep CAP time with slow wave sleep variability time ($r = 0.809$; $P < 1E\text{-}07$). Only the duration of CAP A phases showed a low correlation with the duration of variability events. Finally, the age-related modifications of CAP time and of NREM variability time were found to be very similar. The new method for the automatic analysis of NREM sleep amplitude variability presented here correlates significantly with visual CAP parameters; its application requires a minimum work time, compared to CAP analysis, and might be used in large studies involving numerous recordings in which NREM sleep EEG amplitude variability needs to be assessed.

INTRODUCTION

Cyclic alternating pattern (CAP) (Terzano *et al.*, 1985, 1988, 2001) is a spontaneous rhythm of non-rapid eye movement (NREM) sleep characterized by electroencephalographic (EEG) oscillations corresponding, most probably, to recurrent activation events and unstable sleep depth. Each oscillation or cycle is composed of an EEG transient (phase A of the cycle) separated by intervals of background activity (phase B of the cycle). Three main CAP phase A subtypes have been described on the basis of the corresponding EEG frequency content. Subtype A1 is characterized by prominent EEG synchronized slow waves; subtype A3 by EEG fast rhythms; and subtype A2 on a combination of both (Terzano *et al.*, 2001).

Since its discovery, CAP has been applied extensively to the study of human sleep in normal and pathological conditions in a wide age range, from newborns to adults and older patients (Bruni *et al.*, 2010; Parrino *et al.*, 2011) and is now considered to be a comprehensive tool to study sleep instability (Parrino *et al.*, 2011).

Despite its importance and usefulness in research and clinical work, CAP scoring has not been carried out extensively in the sleep medicine community because it is a time-consuming task, especially in routine clinical work. This can be considered as a primary limiting factor for CAP scoring of

large data sets for research purposes and of clinically important recordings, when a quick evaluation is needed, and also in consideration of the economic aspects of public health management. For this reason, a series of computerized automated tools for the quantification of CAP has been proposed, with different results (De Carli *et al.*, 2004; Ferri *et al.*, 2005a; Navona *et al.*, 2002; Rosa *et al.*, 1999), but none of them has been accepted by the scientific community as the gold standard to score CAP. In addition, as for all visual sleep scorings, a certain degree of inter-rater variability has also been reported for CAP scoring (Ferri *et al.*, 2005a; Rosa *et al.*, 2006).

For these reasons, we decided to arrange a new measure of NREM sleep EEG amplitude oscillations based exclusively on an automatic and quantitative analysis, with a very limited intervention by the scorer. The aim was to obtain a method of measurement of the EEG signal amplitude oscillations during NREM sleep that would correlate with the visually extracted CAP parameters, without necessarily being of the same magnitude. Thus, we did not aim to mimic the visual scoring of CAP but, rather, to arrange a measure correlated with it that can be included easily in the main sleep analysis software tools available on the market and be used in the research and clinical work environments.

SUBJECTS AND METHODS

Subjects and polysomnographic recordings

For this study 98 recordings were collected; these recordings had been obtained from the same number of subjects (50 males and 48 females, mean age 25.4 ± 21.18 years, range 4–80) who had been enrolled previously as normal controls in different studies carried out by our group. All studies were approved by the local Ethics Committees and all subjects gave their informed consent to the procedures. All subjects had regular life routines, did not smoke and did not take any alcohol drink in the 3 days preceding the study. Caffeine was also avoided during the day preceding the study.

All subjects underwent one overnight polysomnographic recording after one adaptation night, which comprised at least three EEG channels (always including C3 or C4, and O1 or O2, referred to A1 or A2), electro-oculogram (EOG) (two channels) and electromyogram (EMG) of the submentalis muscle. Signals were sampled at 200 or 256 Hz and stored on hard disk in European data format (Kemp *et al.*, 1992) for further analysis. EEG signals, in particular, were band-pass filtered digitally at 0.1–50 Hz, 12-bit A/D precision.

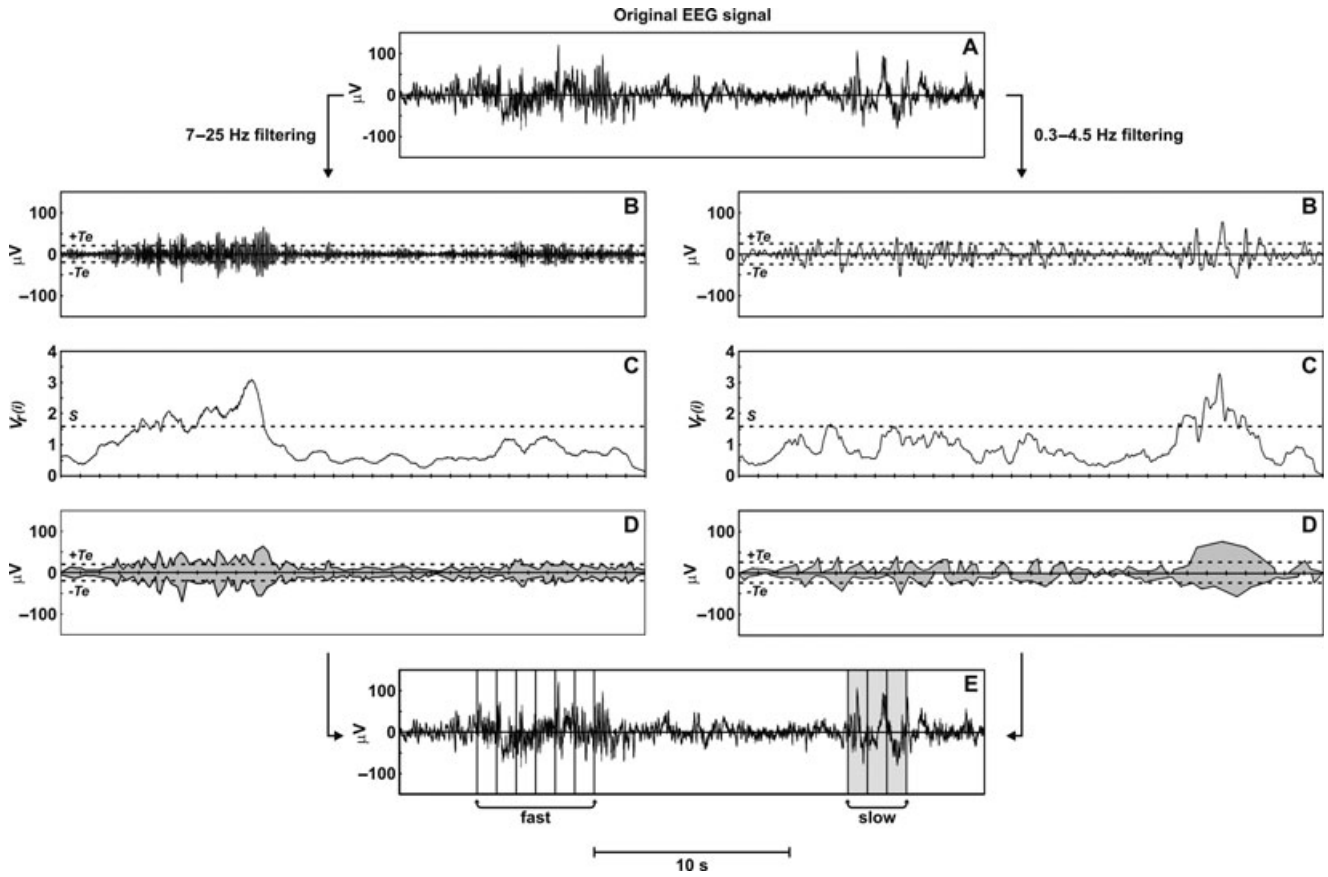

Figure 1. Schematic representation of the non-rapid eye movement (NREM) sleep encephalographic (EEG) amplitude oscillation detection described in this study.

Sleep staging and visual CAP analysis

Sleep stages were scored following standard criteria (Rechtschaffen and Kales, 1968) in 30-s epochs; this scoring served as the basis for scoring CAP. Subsequently, each CAP A phase was detected visually in each recording (on the C3/A2 or C4/A1 derivation) during NREM sleep, and classified into three subtypes (A1, A2 and A3) according to the rules defined by Terzano *et al.* (2001). Visual detection of the CAP A subtypes was carried out by means of the sleep analysis software Hypnolab version 1.3 (SWS Soft, Italy), which allows the scorer to mark manually the events of interest and to assign the desired event subtype. The same software generates a report automatically with a series of CAP parameters which were used for statistical analysis. For this detection, the C3 or C4 channel was used; however, the human scorer takes into account all available channels when scoring CAP.

Automatic analysis of NREM sleep variability

For the automatic analysis of the EEG amplitude oscillations performed in this study, the same sleep staging carried out before the visual CAP analysis was used to identify NREM sleep epochs. Fig. 1 shows a schematic diagram of the detection process.

The first important step of this approach was to determine two absolute threshold values, one for the level of low-frequency (0.3–4.5 Hz) EEG activities and another for high-frequency EEG rhythms (7–25 Hz, with the exclusion of the sigma band by means of a notch filter). The amplitude of EEG high-frequency and low-frequency activities of all events detected had to be higher than these thresholds. Thus, an automatic amplitude threshold setting was carried out by measuring the amplitude of the EEG signal of the same channel used for CAP analysis (C3 or C4) during all available epochs of NREM sleep. The root mean square (RMS) of the signal was computed and the threshold set as 1.5 × RMS. This step was performed twice, after band-pass filtering, for the above two frequency bands of interest (Fig. 1b). The 1.5 multiplication factor indicated above was chosen on the basis of the well-established visual rules for CAP detection that indicate an amplitude threshold for its A phases of 1.5 above baseline (Terzano *et al.*, 2001).

Similarly to one of the CAP detection methods proposed earlier (Navona *et al.*, 2002), we used an approach based on two sliding windows of different length. For all NREM sleep we first analysed the amplitude variability of the EEG signal (separately for each frequency band of interest) in 90-s epochs. Then the same analysis was performed for each second of the 30 central seconds of this big epoch and the ratio between the two variability measures computed (Fig. 1c). The length of these two windows was determined on the basis of the visual rules for scoring CAP (Terzano *et al.*, 2001) and on previous studies on the time structure of CAP (Ferri *et al.*, 2005b, 2006; Smerieri *et al.*, 2007). As an example, in the past we have computed the detrended fluctuation analysis of slow wave activity during sleep and found two scaling regions with the first corresponding to fluctuations with period 0.09–0.75 s and the second to fluctuations with period 1.5–24.0 s (Ferri *et al.*, 2005b). Thus, we chose two time windows for this method that would embed these values.

The mathematical details of this approach and of the following steps are reported in the Appendix. All seconds with a variability measure exceeding 1.6 times (this value was also chosen on the basis of the 1.5 times rule of CAP detection with a subsequent empirical small adjustment) that of the variability within the whole 90-s epoch were marked. Moreover, among these, only the seconds during which the amplitude of the envelope of the filtered original signal passed the threshold value described above (obtained with the measurement of the signal RMS) were retained for the final analysis (Fig. 1d). All these steps were always repeated twice, once for each of the two frequency bands of interest.

The 90-s epochs were shifted along the signal by 30 s and the analysis repeated. At the end of the process, we obtained two series of values, one for each frequency band, indicating single seconds of the recording which were characterized by a signal variability 1.6 times higher than that of the surrounding 90 s and by a signal absolute amplitude 1.5 higher than the overall RMS amplitude of the signal during NREM sleep. These two series of values were used for the

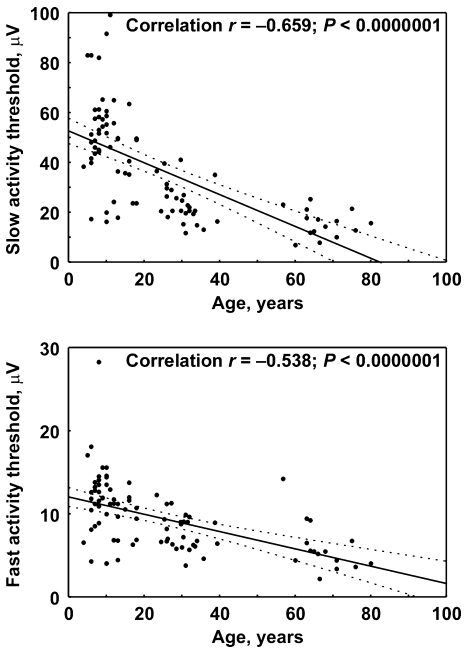

Figure 2. Correlation between the amplitude threshold obtained after the calculation of the root mean square (RMS) value of the encephalographic (EEG) signal during non-rapid eye movement (NREM) sleep for the slow and fast activities and age of the subjects included in this study (n = 98). Also the regression line is shown (continuous), together with the 95% confidence interval lines (dashed).

identification of all sequences of consecutive (at least 2) marked seconds; these sequences will be referred to as 'slow' or 'fast', on the basis of the preponderance of seconds belonging to one or the other EEG bands of interest (Fig. 1e). All isolated (non-sequence) values were discarded.

The whole computation was performed by a routine fully embedded in the same software Hypnolab 1.3 (SWS Soft) that was also used for visual sleep staging and CAP detection (see above).

Statistical data analysis

First, we analysed the correlation between age and the automatic thresholds computed on the basis of the RMS value of the EEG signal during NREM sleep, because a decrease with age of the sleep EEG is expected (Gaudreau *et al.*, 2001).

Moreover, different approaches were used to characterize in detail the statistical aspects of the results of this study. On one hand, the differences between the results of the automatic NREM sleep EEG oscillation analysis and of the visual CAP analysis were compared by means of Student's *t*-test for paired data sets; on the other hand, the correlation between these parameters was assessed by means of Pearson's correlation coefficient. We also computed Kendall's *W* coefficient of concordance which expresses the simultaneous association (relatedness) between different sets of rankings. This statistic is commonly used to assess inter-rater reliability. The range of Kendall concordance is from 0 to 1; values close to zero represent lack of agreement in the rankings of the variables among raters, while values close to 1 represent perfect agreement (Siegel, 1956). Finally, the Bland–Altman plots (Bland and Altman, 1986) were also used for analysis of the agreement between the two methods.

Differences and correlations were considered statistically significant at $P < 0.05$. The commercially available software STATISTICA data analysis software system, version 6 (StatSoft, Inc., Tulsa, OK, USA, 2001) was used for all statistical tests.

RESULTS

First, the correlation between age and the automatic thresholds computed on the basis of the RMS value of the EEG signal during NREM sleep was analysed. Both thresholds showed a clear and significant age-related trend (Fig. 2), with a decrease from childhood to adulthood and older age, which was much more pronounced for the slow wave band (0.3–4.5 Hz) than for the high-frequency EEG band (7–25 Hz).

Corresponding parameters in the two analyses were used for all comparisons and correlations. These parameters are listed in Table 1, which reports the comparison between the results of the automatic NREM sleep EEG oscillation analysis and of the visual CAP analysis. Most of these parameters show highly significant differences, with the automatic method providing higher estimates of NREM sleep EEG oscillations than visual CAP scoring, both in terms of time and percentage, mainly during light sleep (LS, sleep Stages 1 and 2); during slow-wave sleep (SWS, sleep Stages 3 and 4), no differences were found. Additionally, the event duration was clearly shorter in the automatic method than the visual CAP scoring.

Even if two measures can be systematically different they can still be correlated; this was tested by means of Pearson's correlation coefficient, which was found to be significant for all parameters considered but one: the event duration (Table 1). It is important to note that all correlations were positive and were also evaluated visually by drawing the corresponding scatter plots as well as the regression lines (Fig. 3).

An additional parameter was used to test the degree to which the two methods not only correlate but are also able to rank the recordings similarly. This method is Kendall's *W* coefficient of concordance, which was highest for the total number of events and lowest for the automatic EEG oscillation parameters during SWS (Table 1).

Moreover, the Bland–Atman plots were drawn for all the parameters considered, as shown in Fig. 4. It is important to note that these plots are particularly informative for the

Table 1 Comparison between the results of the automatic non-rapid eye movement (NREM) sleep amplitude variability analysis and of the visual cyclic alternating pattern (CAP) analysis. A statistical comparison is reported for corresponding parameters in the two analyses

						Student's *t*-test		*Pearson*'s correlation		*Kendall*'s agreement
b	*Mean*	*SD*	*CAP parameters*	*Mean*	*SD*	*P*	*P<*	*r*	*P<*	*W*
Total events, *n*	567.0	166.29	Total CAP events, *n*	377.3	105.66	−13.427	7.51E-24	0.548	1E-07	0.700
NREM variability time, min	171.1	47.17	Total CAP time, min	139.6	42.96	−7.664	1.38E-11	0.596	1E-07	0.375
NREM variability, %	50.1	9.71	Total CAP rate, %	40.7	12.03	−8.114	1.53E-12	0.461	0.000002	0.426
LS variability time, min	99.9	33.47	LS CAP time, min	71.7	37.67	−8.693	8.85E-14	0.597	1E-07	0.400
LS variability, %	41.5	12.50	LS CAP rate, %	34.9	21.71	−3.165	0.002	0.391	0.000072	0.202
SWS variability time, min	72.7	34.58	SWS CAP time, min	69.3	31.17	−1.619	NS	0.809	1E-07	0.021
SWS variability, %	71.9	20.06	SWS CAP rate, %	68.9	18.57	−1.507	NS	0.522	1E-07	0.025
Event duration, s	3.44	0.29	A phase duration, s	8.2	1.47	31.981	0	0.124	NS	0.010

Variability time: duration of the total number of sleep epochs containing events; percentage: variability time/stage total time × 100; SD, standard deviation; LS, light sleep (sleep Stages 1 and 2); SWS, slow wave sleep (sleep Stages 3 and 4); NS, not significant.

prediction of the possible range of one measurement, given a value of the other. The limits of this range, usually set as the mean difference ± 2 standard deviations (SD) are also referred to as the limits of agreement between the two methods under analysis (Bland and Altman, 1986). These limits appear to be somewhat wide in all instances.

Finally, Fig. 5 depicts the age-related modifications of CAP time (top panel) and of NREM EEG amplitude oscillation time (bottom panel) in the group of subjects included in this study. The two graphs show a very similar peak during adolescence, with another small increase during adulthood/older age for CAP time and a stable low NREM EEG oscillation time during the same age period.

DISCUSSION

The main scope of this study was to obtain a method of measurement of the EEG signal amplitude variability during NREM sleep correlated with CAP parameters, without necessarily being of the same magnitude. This goal was fully achieved, because almost all the parameters considered showed good correlation with the corresponding CAP measures. This allows us to propose this new method for a series of potential applications in the future, including the evaluation of NREM sleep amplitude variability in large clinical trials. This is particularly important and, as an example, CAP analysis has not been included in the new American

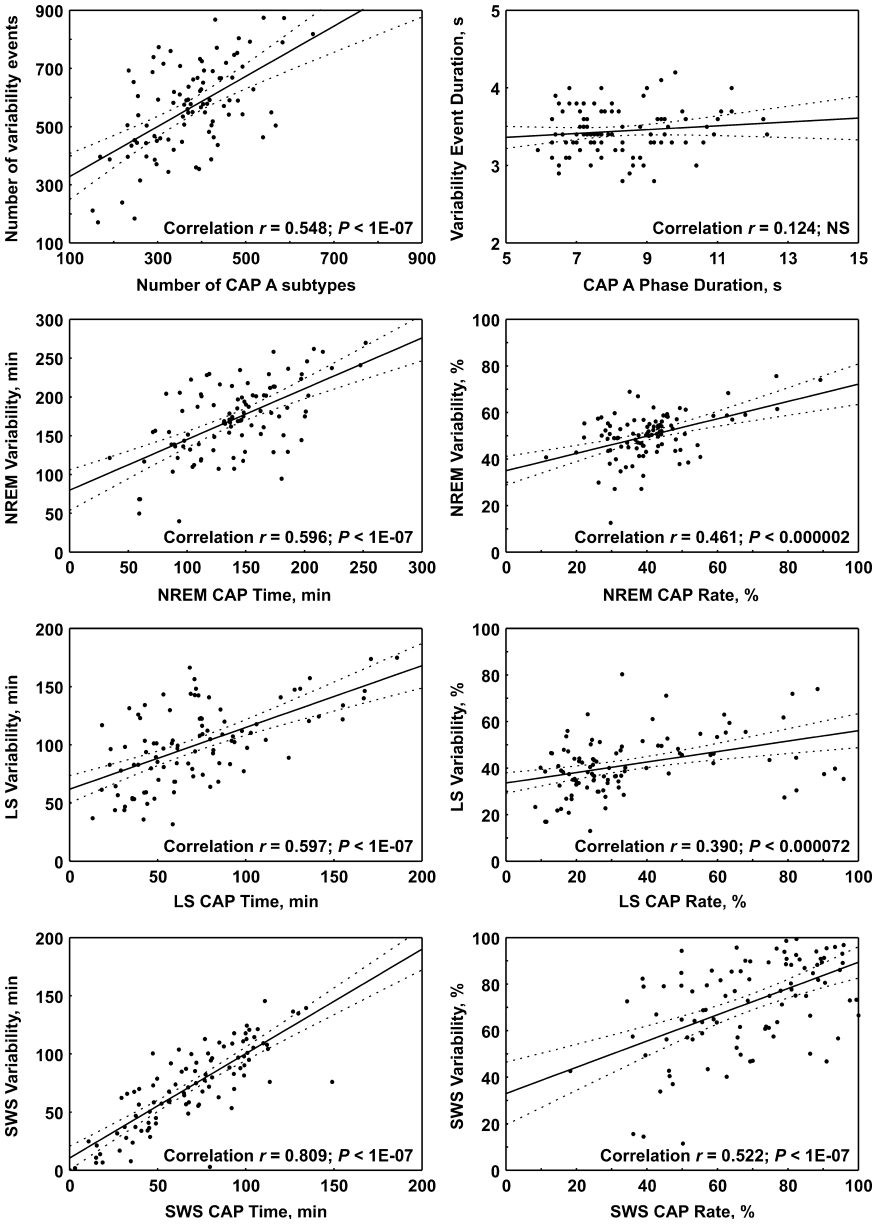

Figure 3. Correlation between different automatic sleep encephalographic (EEG) amplitude variability parameters and their corresponding visual cyclic alternating pattern (CAP) parameters in the group of subjects included in this study (n = 98). Also the regression line is shown (continuous), together with the 95% confidence interval lines (dashed).

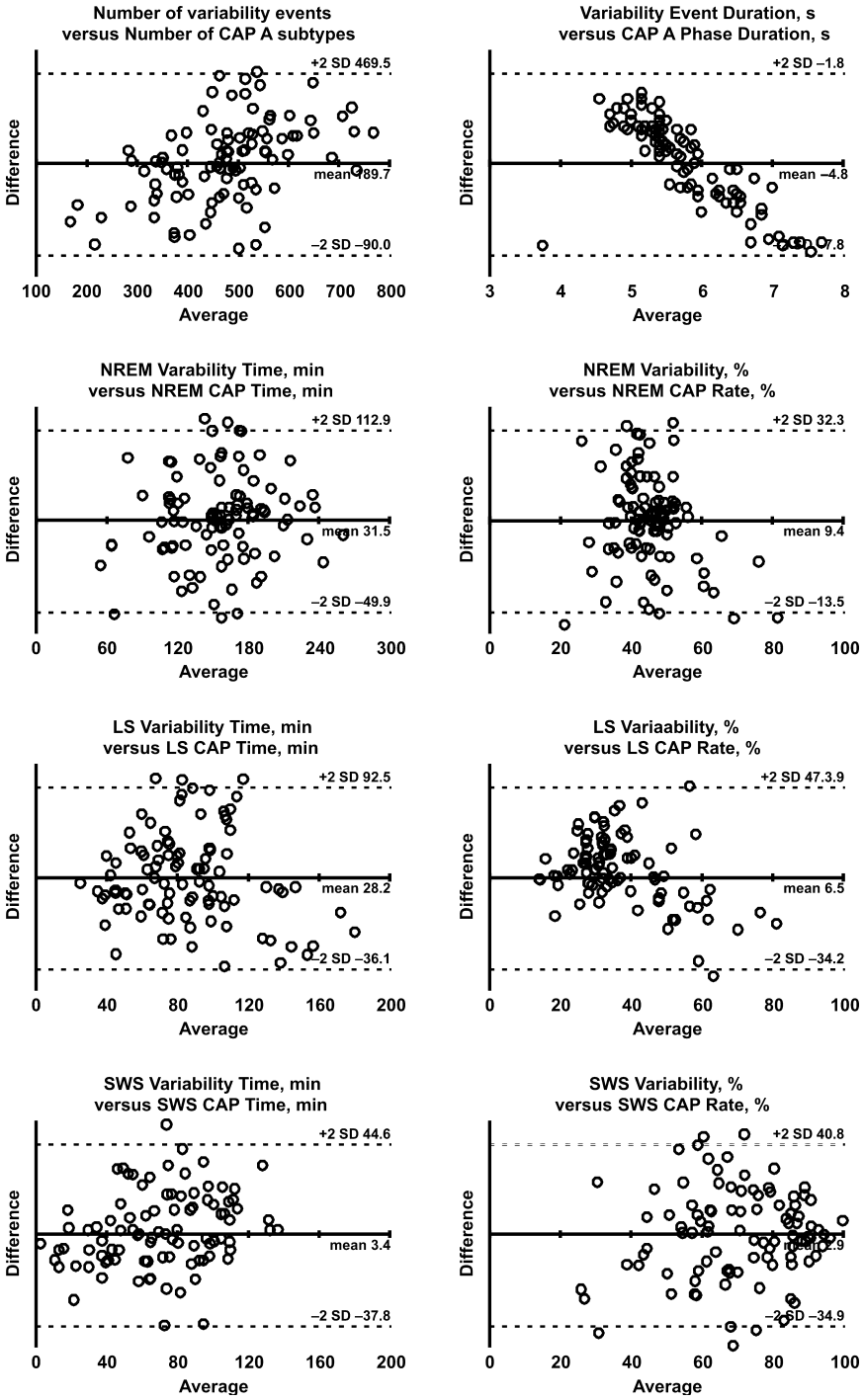

Figure 4. Bland-Altman plots for different automatic sleep encephalographic (EEG) amplitude variability parameters and their corresponding visual cyclic alternating pattern (CAP) parameters in the group of subjects included in this study (n = 98).

Academy of Sleep Medicine (AASM) manual for sleep scoring (Iber *et al.*, 2007) because it was judged to lack a sufficient number of studies with levels 1 or 2 of evidence (Sackett, 1993). This was due mainly to the difficulty in scoring CAP in large numbers of recordings in well-controlled trials; some studies with this level of evidence have now been published (Ozone *et al.*, 2008; Svetnik *et al.*, 2010), but it is unlikely that their number will grow quickly in the near future for the same reasons. The use of an automated quantitative method such as ours might overcome the difficulties and the costs (also in terms of time) needed for visual CAP scoring and in many instances might represent a valid alternative.

It should be also considered that our new method does not substitute fully for the visual CAP analysis because it provides a smaller number of parameters and detects more numerous events with a significantly shorter mean duration.

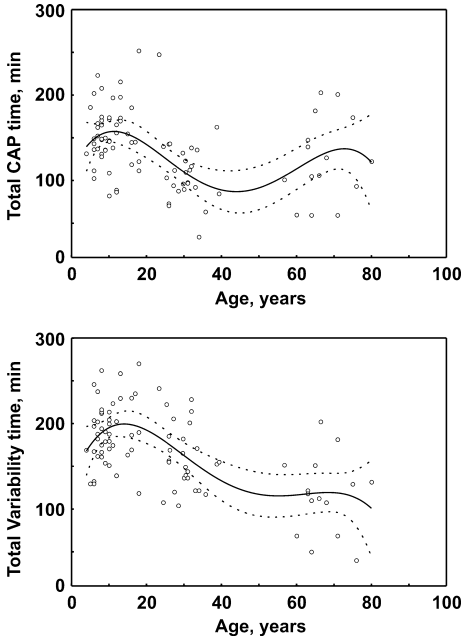

Figure 5. Age-related modifications of cyclic alternating pattern (CAP) time (top panel) and of nonrapid eye movement (NREM) sleep encephalographic (EEG) amplitude variability time (bottom panel) in the group of subjects included in this study (n = 98). Also the quartic polynomial fitting line is shown (continuous), together with the 95% confidence interval lines (dashed).

Consequently, it tends to indicate a higher amount of EEG amplitude oscillations than CAP. We suggest that, when careful analysis of the relationship between fast and slow events or analysis of the time structure of sleep EEG amplitude variability is needed (such as in individual clinical evaluations), visual CAP scoring should be considered because of its superior adequacy for this type of study.

We should take into account that CAP analysis is based on visual scoring, and it might miss some short-term variations of the EEG amplitude that can be better detected using an automated method. Therefore, we do not know if visual CAP analysis detects all the significant amplitude variations of the EEG signal and it cannot be excluded that this new automated method is more sensitive than CAP, and might provide a better picture of NREM sleep EEG amplitude oscillations than visual analysis. This also seems to be in agreement with very recent data obtained by the computation of Hjorth descriptors of NREM sleep EEG (Mariani *et al.*, 2011).

Besides some similarity with a method reported earlier, because of the use of two sliding windows (Navona *et al.*, 2002) our approach is simpler overall and has several new features, including the automatic amplitude threshold setting, the use of a direct measure of amplitude variability, the computation of the envelope of the signals and the use of a double threshold method, etc.

Our new method presents some important strengths. It is almost completely automatic, thus inter-rater variability is greatly reduced (Ferri *et al.*, 2005a); the results are obtained in a fraction of the time needed for visual CAP scoring; the algorithm is fully disclosed in this paper in order to allow companies developing sleep recording and analysis systems to include it in their products; it has been validated in a relatively large group of subjects (n = 98), which is significantly larger than the groups used in previous attempt of automatic CAP scoring (De Carli *et al.*, 2004; Ferri *et al.*, 2005a; Navona *et al.*, 2002; Rosa *et al.*, 1999); it has been validated on a group of subjects with a wide age range (from 4 to 80 years); and the results have been compared extensively with those of visual CAP analysis with full disclosure of their relatedness and differences.

All these considerations support the conclusion that our new algorithm can be used reliably in large studies involving numerous recordings in which NREM sleep EEG amplitude variability needs to be assessed.

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APPENDIX

Automatic amplitude threshold setting

As a first step, an automatic amplitude threshold setting was carried out by measuring the amplitude of the EEG signal of the same channel used for CAP analysis (C3 or C4) during all available epochs of NREM sleep. The root mean square (RMS) of the signal was computed and the threshold set as 1.5× RMS. This step was performed twice, after band-pass filtering, for the two frequency bands of interest, one for the analysis of NREM sleep instability in the slow EEG frequencies (0.3–4.5 Hz) and another for the analysis of instability in the high EEG frequencies (7–25 Hz, with the exclusion of the sigma band by means of a notch filter). In this way, we obtained two threshold values (*Te*) for each subject, which were used for the subsequent analysis; in fact, all events detected had to be higher than this threshold to be confirmed (see below).

Automatic computation of the EEG amplitude variability

We defined the EEG signal as $x(k)$ with $k = 1,2, \ldots, N$; where N represents the number of samples in 90 s. The choice of 90 s was due to the fact that, similarly to CAP, we expected to find events no longer than 60 s (Ferri *et al.*, 2006); using 90-s epochs we assured to have both activation and baseline in all segments analysed. We then computed the variability of the signal in 90 s :

$$V = \frac{\sum_{k=1}^{N} |x(k) - \bar{x}|}{N} \tag{1}$$

with:

$$\bar{x} = \frac{\sum_{k=1}^{N} x(k)}{N} \tag{2}$$

With the signal sampling rate F_c we calculated the instantaneous variability $V_{\text{sec}}(i)$ for all samples in the central 30 s, in mini epochs of 1 s in duration:

$$V_{\text{sec}}(i) = \frac{\sum_{k=-F_c/2}^{F_c/2} |x(i+k) - \bar{x}_i|}{F_c} \qquad i = 30F_c + 1, \ldots, 60F_c \tag{3}$$

where:

$$\bar{x}_i = \frac{\sum_{k=-Fc/2}^{F_c/2} x(i+k)}{F_c} \tag{4}$$

For each sample i the relative variability $V_r(i)$ was calculated as:

$$V_r(i) = \frac{V_{\text{sec}}(i)}{V} \qquad i = 30F_c + 1, \ldots, 60F_c \tag{5}$$

Given a predetermined value $S = 1.6$, we indicated with N_s the number of samples for which the values of $V_r(i)$ was below S and computed the variability V_s for all samples below such a value:

$$V_S = \frac{\sum_{k=1}^{N_s} |x_s(k) - \bar{x}_s|}{N_s} \tag{6}$$

where:

$$x_s(k) = x(k) \quad \forall k \in \{30F_c + 1, \ldots, 60F_c : V_r(k) < S\} \tag{7}$$

and

$$\bar{x}_s = \frac{\sum_{k=1}^{N_s} x_s(k)}{N_s} \tag{8}$$

We then recalculated the relative variability of samples with a previous variability below this threshold:

$$V_{rs}(i) = \frac{V_{\text{sec}}(i)}{V_s} \qquad i = 30F_c + 1, \ldots, 60F_c \tag{9}$$

Subsequently, we indicated with $V_{\text{INST}}(j)$ the vector containing the values of event detection, we also defined $N_c(j)$ as the number of samples with a relative variability $V_{rs}(i)$ above the threshold in 1-s mini epochs; thus we had:

$$V_{\text{INST}}(j) = \begin{cases} 0 & N_c(j) < F_c/2 \\ 1 & N_c(j) \geq F_c/2 \end{cases} \qquad j = 1, 2, \ldots, 30 \tag{10}$$

In this way, we obtained a vector V_{INST} (j) containing 30 values indicating the presence or absence of an event, second by second, in the central 30 s of the input signal $x(k)$.

In order to improve the signal variability detection algorithm, we then performed an additional computation to take into account the absolute amplitude of the signal, as it is probably perceived by the human eye. In particular, we generated the envelope of the central 30 s of the input signal $x(k)$ by means of the Hilbert transformation and performed a smoothing of such an envelope. Finally, we carried out a comparison between the envelope and a predetermined amplitude threshold T_e, previously obtained automatically (see above) on the basis of the RMS value of the entire input signal during NREM sleep, to confirm or not the mini epochs found before with V_{INST} (j) = 1.

We defined *z(i)* as the vector containing the central 30 s of th input EEG signal $x(k)$:

$$z(i) = x(i + 30F_c) \qquad i = 1, 2, \ldots, 30F_c \tag{11}$$

We then generated the envelope of $z(i)$ by means of the Hilbert transform. We indicated with *F[]* the operator providing the Fourier transform of a signal and defined $m(k)$ as:

$$m(k) = \begin{cases} 1 & k = 1, N/2 + 1 \\ 2 & k = 2, 3, \ldots, N/2 \\ 0 & k = N/2 + 2, \ldots, N \end{cases} \tag{12}$$

where N ($=30F_c$) was the number of samples in 30 s.

We obtained $p(k)$ as a complex signal from the following product:

$$\begin{cases} Re\{p(k)\} = m(k)Re\{F[z(k)]\} \\ Im\{p(k)\} = m(k)Im\{F[z(k)]\} \end{cases} \qquad k = 1, 2, \ldots, N \tag{13}$$

By performing the inverse Fourier transform F^{-1}[] of $p(k)$, we obtained the Hilbert transform of $z(k)$:

$$H(k) = F^{-1}[p(k)] \tag{14}$$

The envelope of the signal $z(k)$ was given by:

$$y(k) = \sqrt{Re\{H(k)\}^2 + Im\{H(k)\}^2} \tag{15}$$

We subsequently performed a smoothing of the envelope obtained above:

$$y_s(k) = \frac{\sum_{j=1}^{\frac{M-1}{2}} \left(\frac{M+1}{2} - j\right)[y(k-j) + y(k+j)] + \frac{M+1}{2} y(k)}{\left(\frac{M+1}{2}\right)^2}$$
$$\frac{M+1}{2} \le k \le N - \frac{M-1}{2} \tag{16}$$

where N was the number of samples of the signal and M a fixed smoothing parameter F_c/2-1.

At this point, we reconsidered 1-s mini epochs with V_{INST} (j) = 1:

$$V_{INST}(j) = \begin{cases} 1 & \bar{y}_s(j) \ge T_e \\ 0 & \bar{y}_s(j) < T_e \end{cases} \quad j = 1, 2, \ldots, 30 \text{ and } V_{INST}(j) = 1 \tag{17}$$

where:

$$\bar{y}_s(j) = \frac{\sum_{k=(j-1)F_c+1}^{jF_c} y_s(k)}{F_c} \qquad j = 1, 2, \ldots, 30 \tag{18}$$

Finally, the following recomputation of the detection vector $V_{INST}(j)$ was performed:

$$V_{INST}(j) = \begin{cases} 1 & V_{INST}(j-1) = V_{INST}(j+1) = 1 \\ 0 & V_{INST}(j-1) = V_{INST}(j+1) = 0 \end{cases}$$
$$j = 2, 3, \ldots, 29 \tag{19}$$

$$V_{INST}(j-2) = V_{INST}(j-1) = V_{INST}(j+2) = V_{INST}(j+3) = 1 \rightarrow V_{INST}(j) = V_{INST}(j+1) = 1 \tag{20}$$

As already performed for the computation of the RMS values of the EEG signal in different frequency bands, the whole process described above was performed twice, after band-pass filtering, for the two frequency bands of interest: one for the analysis of the slow EEG frequencies (0.3–4.5 Hz) and another for the analysis of the high EEG frequencies (7–25 Hz, with the exclusion of the sigma band by means of a notch filter). In this way, we obtained two detection vectors, which were used for the subsequent definition of slow or fast events $V_{SLOW}(j)$ and $V_{FAST}(j)$.

Detection of EEG amplitude variability events and calculation of the parameters

We proceeded with the combination of the two vectors obtained at the end of the previous step by obtaining a single vector containing:

$$V_{INST}(j) = \begin{cases} 0 & V_{SLOW}(j) = V_{FAST}(j) = 0 \\ 1 & V_{SLOW}(j) = 1; V_{FAST}(j) = 0 \\ 2 & V_{SLOW}(j) = V_{FAST}(j) = 1 \\ 3 & V_{SLOW}(j) = 0; V_{FAST}(j) = 1 \\ & j = 1, 2, \ldots, N \end{cases} \tag{21}$$

This new vector was used for the identification of all sequences of consecutive $V_{INST}(j) > 0$ (at least 2); these sequences have been referred to as 'events' in this paper and were classified as 'slow' if their average value of $V_{INST}(j)$ was ≤2 or 'fast' if it was >2. All isolated $V_{INST}(j) > 0$ mini epochs were discarded.