The individual adjustment method of sleep spindle analysis: Methodological improvements and roots in the fingerprint paradigm

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A R T I C L E   I N F O

Article history:
Received 30 August 2008
Received in revised form 8 November 2008
Accepted 10 November 2008

Keywords:
Polysomnography
Electroencephalogram
Cortical synchronization
Digital signal processing
Sleep spindles
Automated pattern recognition

A B S T R A C T

Evidence supports the robustness and stability of individual differences in non-rapid eye movement (NREM) sleep electroencephalogram (EEG) spectra with a special emphasis on the 9–16 Hz range corresponding to sleep spindle activity. These differences cast doubt on the universal validity of sleep spindle analysis methods based on strict amplitude and frequency criteria or a set of templates of natural spindles. We aim to improve sleep spindle analysis by the individual adjustments of frequency and amplitude criteria, the use of a minimum set of a priori knowledge, and by clear dissections of slow- and fast sleep spindles as well as to transcend the concept of visual inspection as being the ultimate test of the method’s validity. We defined spindles as those segments of the NREM sleep EEG which contribute to the two peak regions within the 9–16 Hz EEG spectra. These segments behaved as slow- and fast sleep spindles in terms of topography and sleep cycle effects, while age correlated negatively with the occurrence of fast type events only. Automatic detections covered 92.9% of visual spindle detections (A&VD). More than half of the automatic detections (58.41%) were exclusively automatic detections (EADs). The spectra of EAD correlated significantly and positively with the spectra of A&VD as well as with the average (AVG) spectra. However, both EAD and A&VD had higher individual-specific spindle spectra than AVG had. Results suggest that the individual adjustment method (IAM) detects EEG segments possessing the individual-specific spindle spectra with higher sensitivity than visual scoring does.

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1. Introduction

Sleep spindles are usually defined as groups of 12–15 Hz sinusoidal electroencephalogram (EEG) waves occurring mainly during stage 2 non-rapid eye movement (NREM) sleep but occasionally appearing in stages 3 and 4 sleep as well (De Gennaro and Ferrara, 2003). There is growing neurophysiological knowledge regarding the nature of neural mechanisms generating sleep spindles, suggesting the role of hyperpolarization-rebound sequences in thalamocortical relay cells triggered, grouped and synchronized by cortico-cortical networks (Steriade, 2003). Moreover, there is a high-degree of interindividual difference in sleep spindle features accompanied by a remarkable intraindividual (night-to-night) stability (Silverstein and Levy, 1976; Gaillard and Blois, 1981; Werth et al., 1997; Tan et al., 2000; De Gennaro et al., 2005). The NREM sleep EEG power spectra at the 8 to 16 Hz frequency covering alpha and spindle activities is characterized by an individual profile, which is stable over time, resistant to experimental perturbations and strongly influenced by genetic factors (De Gennaro et al., 2008). A distinction of slower and faster sleep spindles based on frequency and topography was given by Gibbs and Gibbs (1950) and confirmed by studies using modern techniques like low-resolution electromagnetic tomography (Anderer et al., 2001), magnetoencephalography (Urakami, 2008), electrocorticography (Nakamura et al., 2003) and functional magnetic resonance imaging (Schabus et al., 2007). The frequency of slow spindles mostly corresponds to the alpha frequency range and detailed EEG studies suggest the possibility that slow spindles are anterior peaks of alpha activity during NREM sleep (De Gennaro and Ferrara, 2003). Given these evidences it is quite surprising that most of the methods of sleep spindle analysis are ultimately still based on, validated by, and tied to visual detection of spindles performed by experienced human scorers. By accepting visual scoring as the final test of automatic sleep spindle analysis one is implicitly assuming that human pattern recognition capacities are still superior to computer-based methods of spindle detection or that modern neurophysiological knowledge did not influence the definition of sleep spindles. Since we do not agree with these assumptions, we developed an improved method of sleep spindle analysis, which is a modified version of our previously published one (Bódizs et al., 2005). Our main starting points in the development of our method were the following:
1. Sleep spindles are groups of waves in the 9–16 Hz range lasting at least 0.5 s and appearing in NREM sleep EEG records (De Gennaro and Ferrara, 2003; De Gennaro et al., 2005).

2. The exact spectral content of sleep spindles is individual-specific. In humans it emerges as an individually stable trait-like feature characterized most often by two distinct spectral peaks with different topography and sleep cycle dynamics (Werth et al., 1997; De Gennaro et al., 2005; Buckelmüller et al., 2006). There is exceptionally low channel-by-channel, cycle-by-cycle and night-by-night variation in the individual-specific frequency bands delimiting the slow- and fast sleep spindles (Werth et al., 1997).

Based on these statements we defined sleep spindles as those segments of the NREM sleep EEG which last at least 0.5 s and contribute to one of the two individual-specific spectral peaks observed in the 9–16 Hz range. By accepting this definition our aim was to:

1. define the individual-specific spectral peaks in the 9–16 Hz range;
2. calculate the individual- and derivation-specific amplitude criteria for the two spindle types separately;
3. perform an adequate band-pass-filtering and to obtain the precise envelope curves in the individual-specific frequency ranges;
4. obtain not only the density, but also the mean amplitude and mean duration of the two sleep spindle types for each subject and each EEG derivation;
5. validate our method by testing previously established relationships:
   (a) topographical difference between slow- and fast sleep spindles (anterior versus posterior predominance of slow- and fast sleep spindles, respectively);
   (b) declining trend of slow spindling and increasing trend of fast spindling over consecutive sleep cycles;
   (c) drop of sleep spindles with ageing and its interaction with sleep cycle effects;
6. compare the output of our automatic sleep spindle detection technique with the visual procedure performed by a trained expert.

Our previously published method (Bódizs et al., 2005) was modified in accordance with our sleep spindle definition and with new developments in the field. As regarding methodological improvements we introduced the zero-padding of EEG segments prior to fast Fourier transformation (FFT), as this was shown to be a reliable method of estimating the dominant spindle frequencies (Huupponen et al., 2006). Moreover, band-pass-filtering was based on Gauss-filters instead of Butterworth ones. And lastly we did not introduce any ad hoc correction in the amplitude criteria, but calculated a precise envelope of the filtered signals.

We hypothesized that the individual adjustment method (IAM) of sleep spindle analysis, which is an operationalization and application of our sleep spindle definition on human sleep EEG records:

1. results in spindle detections, which behave as slow- and fast sleep spindles in terms of topography, sleep cycle effects and age;
2. results in sleep spindles with an individual-specific spectral content paralleling the individual fingerprints of sleep EEG spectra, but being also articular in terms of the individual-specific spectral peaks;
3. is much more sensitive than visual detection performed by human experts (detect more spindles than humans), but the extra-spindles detected by the IAM:
   (a) share the individual-specific spectral content unlike those visually detected spindles which are not covered by IAM;
   (b) are characterized by an amplitude spectrum exceeding the average amplitude spectrum of the whole EEG segment (spindle + no-spindle) in terms of individual-specific spindle-peaks unlike those visually detected spindles which are not covered by IAM.

2. Methods

2.1. Subjects and procedures

Polysomnographic recordings of 46 adult subjects (29 men and 17 women; mean age: 32.46 years; age range: 17–55) participating in two different research projects were used as a database for the current research. The research protocols were approved by the Ethical Committee of the Institute of Behavioural Sciences Semmelweis University Budapest. All subjects signed informed consent for the participation in the studies. According to a semi-structured interview subjects were healthy and free of any current drug effects including contraceptives. Small and habitual doses of nicotine and caffeine, but not alcohol were allowed. Results of the Edinburgh Handedness Inventory revealed 41 right-handed, three left-handed and two ambidextrous subjects in our database. The timing of lights off was determined by subjects’ habit, and the awakenings were spontaneous. Sleep was recorded for two consecutive nights by standard polysomnography, including electroencephalography (recording sites: Fp1, Fp2, F3, F4, Fz, F7, F8, C3, C4, Cz, P3, P4, T3, T4, T5, T6, O1, and O2), left- and right electro-oculography (EOG), bipolar submental electromyography (EMG), electrocardiography (ECG), as well as abdominal and thoracic respiratory movements.

EEG electrodes were referred to the contra lateral mastoid. We used the right mastoid as a reference for the midline EEG electrodes. Impedances for the EEG electrodes were kept below 5 kΩ.

Signals were collected, pre-filtered, amplified and digitized at a sampling rate of 249 Hz/channel by using the 30 channel Flat Style SLEEP La Mont Headbox with implemented second order filters at 0.5 Hz (high pass) and 70 Hz (low pass) as well as the HBX32-SLP 32 channel preamplifier (La Mont Medical Inc. USA). An additional 50 Hz digital notch filtering performed by the DataLab acquisition software (Medcare, Iceland) was carried out before data analysis. Wakefulness and sleep stages of the second night recordings were identified manually by experienced scorers according to the standardized criteria (Rechtschaffen and Kales, 1968). Epochs containing artifacts detected by visual inspection of the EEG, EMG, EOG and ECG signals were excluded from further analyses.

2.2. Spindle analysis

The steps of sleep spindle analysis were the following:

1. Calculating the average amplitude spectra of the NREM sleep records: in 4 s Hanning-corrected (50%) epochs zero-padded to 4096 points (16.45 s) resulting in amplitude spectra with 0.06 Hz spectral resolution. The amplitude spectrum is the normalized magnitude of the FFT of the signal. That is a sine wave of amplitude A and frequency F yields a Spectrum of amplitude A at frequency F.

2. Obtaining the boundary frequencies for spindles. This was performed by down-sampling the 9–16 Hz amplitude spectra of each channel by a factor of 4 (in order to avoid small fluctuations in convex and concave periods), then calculating the second order derivatives of these spectra and averaging them over channels (see the very low channel-by-channel variation in spectral peak frequencies in Werth et al., 1997). The zero-crossing points encompassing the two largest (negative) peaks are the frequency boundaries of slow- and fast sleep spindles. If selection
of the appropriate peaks is not unequivocal decision is based on visual inspection of the spectra of anterior (slow-spindle dominated) and posterior (fast-spindle dominated) channels. The zero-crossing points are rounded to the closest bins within the high-resolution (0.06 Hz) spectra.

3. Obtaining the amplitude criteria of slow- and fast sleep spindles: spindles are defined as those EEG segments contributing to the peak region of the spectrum. Hence we intended to obtain an amplitude criteria corresponding to the line determined by the $y$-values of the zero-crossing points in the spectrum. As this is a linear interpolation in the amplitude domain, the number of frequency bins falling between the frequency boundaries of slow- and fast sleep spindles were multiplied with the mean of the $y$-values (expressed in micro-Volts) corresponding to the frequency boundaries of the spectrum. This was done individually for each channel and based on the spectra of 0.06 Hz resolution.

4. Obtaining the envelope of the rectified slow- and fast sleep spindle activity: EEG channels are bandpass filtered for slow- and fast sleep spindle frequencies by multiplying their 4096 point FFTs with the Gaussian function $f(x) = e^{(x-x_0)/w^2}$, where $x$ varies between zero and the Nyquist frequency according to the spectral resolution, $x_0$ is the middle frequency of the spindle range, and $w$ is the width of the spindle range. The resulting products are inverse Fourier transformed, rectified, filtered with a 22-points Hanning-weighted moving average, and multiplied by $\pi/2$, which is the inverse of the average of a rectified sine wave. Given the narrow frequency ranges and the resulting near-perfect sinusoidal wave in the filtered signal this procedure issues in a particularly reliable envelope of the spindle activities (Fig. 1).

5. Detection of sleep spindles: where the envelopes of the rectified signals exceed the amplitude criteria for more than 0.5 s slow- or fast sleep spindles are detected according to the previously adjusted frequency criteria (Fig. 1).

6. Calculation of spindle features: spindle density is the ratio of the number of detected spindles by the length in minutes of the EEG record in which they occurred. Average spindle amplitudes are the average amplitudes of the rectified signals in the midpoint of the detected spindles. Average spindle durations are average duration of those segments in which the envelopes of the rectified signals exceed the amplitude criteria for more than 0.5 s.

We performed the above calculations for the first four periods of NREM sleep, including stages 2, 3 and 4 hence we obtained cycle-by-cycle spindle-data for all subjects.

2.3. Validation by topography, sleep cycle- and age effects

Slow spindles are known to be relatively enhanced in anterior (frontal) as compared to posterior (parietal) channels, while the opposite is true for fast sleep spindles (Zeitlhofer et al., 1997). In order to test the validity of our sleep spindle analysis method we compared the measured characteristics of sleep spindles between the anterior and posterior channels.

Consecutive NREM sleep episodes are characterized by a declining trend in slow sleep frequency activity and an increasing trend in high spindle frequency activity (Werth et al., 1997). Based on this assumption we compared the spindle characteristics of the first four NREM sleep episodes.

Ageing is characterized by a decrease in sleep spindling, which is particularly strong in the third and fourth sleep cycle (Landolt et al., 1996). Therefore we also tested the effects of age on sleep spindles analyzed by our method. In this procedure we used the spindle data of four recording sites: F3, P3, F4 and P4.

2.4. Comparison with visual detection

In order to compare the IAM with visual detection of sleep spindles, there was selected a 15–20 min long artifact-free EEG segment from the record following the first 10 min of NREM sleep of the third sleep cycle in a subsample of 12 subjects. One of the authors (RB) performed the visual detection of sleep spindles in channels F4-A1 and P4-A1 separately. The results of visual spindle scoring were compared with the results of the automatic one in terms of match-mismatch, overlap, correlation and spectral content. Criteria for the visual detection were the following: a group of 9–16 Hz waves lasting at least 0.5 s. During the visual procedure a mark was set at both the beginning and the end of the individual sleep spindles. A match with automatic detection supposed at least one common point in the results of the two methods (IAM and visual) of spindle detections. Overlap was measured with number of common points and expressed in percents. By correlation analysis we intended to compare visual scoring and IAM with respect to their outcome of measuring individual differences in sleep spindling. Spectral features of the whole EEG segment, as well as of the exclusively automatic detections (EADs), exclusively visual detections, matching automatic-and-visual detections, and segments with no spindle detections were determined by average amplitude spectra based on mixed-radix FFTs of the corresponding segments. As the spectra of segments with different length were of different resolution we maximized the length of the analyzed segments in 2048 points. Segments longer than 2048 points were raveled to 2048 points, while the amplitude spectra of the shorter ones were interpolated to 2048 points by using the zero-insertion FFT procedure. The parallelism of the spectra was tested by Pearson-product moment correlations, while spectral contents according to the individual-specific spindle ranges were compared by analysis of variance.

Signal processing was performed with DADiSP2002 (DSP Development Corp. USA), while statistical analyses with STATISTICA 7.1 (Statsoft Inc. USA).
showed that primarily the fast type of sleep spindles were characterized by a left hemispheric dominance.¹ The main effect of the topography factor revealed an overall higher spindle density over the parietal regions, as compared to the frontal ones (F = 59.42; d.f. = 1, 37; p < .000001). Topography interacted with spindle type (F = 121.13; d.f. = 1, 37; p < .000001) pointing to a higher fast sleep spindle density in the parietal as compared to the frontal derivations (Tukey HSD test: p = .000167) as well as to a reverse topographic pattern in slow spindling (Tukey HSD test: p = .037). Over and above, there emerged more slow than fast sleep spindles in the frontal channels (Tukey HSD test: p = .000167), as well as more fast than slow spindles in the parietal channels (Tukey HSD test: p = .000167), although the factor sleep cycle did not produce a significant main effect (F = 0.49; d.f. = 3, 111; p = .68), nor an interaction with other factors (p > .05 for all combinations), the difference between the first and the fourth sleep cycle regarding the parietally measured fast spindle density proved to be significant. This was also true for the difference between the first and third as well as the first and fourth sleep cycles regarding the frontally measured slow sleep spindle density (Fig. 2). These results suggest a slight increasing trend in the density of the parietally measured fast sleep spindles and the opposite in the frontally measured slow ones. As regarding age effects, fast but not slow spindle density correlated negatively with age in cycle 3 and 4 (Table 2).

The same statistical model was applied for the analysis of spindle duration. A significant main effect of the hemisphere (F = 8.04; d.f. = 1, 37; p = .007358) as well as its interaction with spindle type (F = 6.75; d.f. = 1, 37; p = .013262) revealed the pattern already described for spindle density; fast sleep spindle duration showed a consistent hemispheric asymmetry with a left hemispheric dominance. Overall spindle duration was higher in parietal derivations as compared to the frontal ones (F = 5.86; d.f. = 1, 37; p = .02042). As in the case of spindle density this effect was accompanied by an interaction between topography and spindle type (F = 111.13; d.f. = 1, 37; p < .000001), which according to post hoc Tukey HSD tests pointed to higher fast sleep spindle durations in parietal as compared to frontal derivations (p = .000167) as well as to a reverse topographic pattern in slow spindle durations (p = .000168). However, there was

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Spi1-Lower</th>
<th>Spi1-Upper</th>
<th>Spi2-Lower</th>
<th>Spi2-Upper</th>
</tr>
</thead>
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<tr>
<td>NREM 1</td>
<td>10.76 (0.77)</td>
<td>11.60 (0.77)</td>
<td>12.97 (0.58)</td>
<td>14.12 (0.55)</td>
</tr>
<tr>
<td>NREM 2</td>
<td>10.76 (0.81)</td>
<td>11.60 (0.80)</td>
<td>12.86 (0.59)</td>
<td>14.00 (0.59)</td>
</tr>
<tr>
<td>NREM 3</td>
<td>10.76 (0.75)</td>
<td>11.60 (0.80)</td>
<td>12.90 (0.63)</td>
<td>14.07 (0.65)</td>
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<tr>
<td>NREM 4</td>
<td>10.95 (0.86)</td>
<td>11.74 (0.84)</td>
<td>13.06 (0.63)</td>
<td>14.26 (0.68)</td>
</tr>
</tbody>
</table>

¹ In order to test the possible relationship between handedness and spindle dominance spindle asymmetry indexes of average spindle measures (cycles 1–4) were calculated according to the (L – R)/(L + R) formula. None of these indexes (slow and fast spindle density, duration and amplitude in frontal and parietal regions) correlated significantly with the results of the Edinburgh Handedness Inventory (data not reported).
longer lasting slow than fast spindles in both frontal \((p = 0.000167)\) and parietal \((p = 0.000167)\) derivations. Thus, unlike density duration of spindles varied as a function of their type (see Fig. 3): slow spindles were significantly longer lasting than fast ones \((F = 27.53; d.f. = 1, 37; p = 0.000007)\).

No significant main effects of sleep cycles emerged \((F = 2.12; d.f. = 3, 111; p = 0.10096)\) for spindle duration. However, we observed an interaction between sleep cycles and topography \((F = 4.19; d.f. = 3, 111; p = 0.007)\) denoting longer lasting parietal than frontal spindles during the fourth sleep cycle \((p = 0.0259)\). The post hoc analysis of spindle duration according to sleep cycles, topography and spindle type revealed a decrease of slow spindle durations in cycle three and a significant increase in cycle four (both compared to cycle 1). This was true for both frontal and parietal derivations (Fig. 3). The increase in fast sleep spindle duration began from the third sleep cycle and was found in the parietal derivations only (Fig. 3). Spindle duration behaved like spindle density in terms of age effects: the mean duration of fast but not slow spindles in cycles 3 and 4 correlated negatively with age (Table 2).

Table 2 Pearson correlation coefficients pointing to the relationship between sleep spindle measures and age.

<table>
<thead>
<tr>
<th>Density</th>
<th>Duration</th>
<th>Amplitude</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Slow</td>
<td>Fast</td>
</tr>
<tr>
<td>NREM 1</td>
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</tr>
<tr>
<td>F4</td>
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<tr>
<td>F4</td>
<td>-.07</td>
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<tr>
<td>F3</td>
<td>-.14</td>
<td>-.14</td>
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<tr>
<td>P3</td>
<td>-.13</td>
<td>-.12</td>
</tr>
<tr>
<td>NREM 2</td>
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</tr>
<tr>
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<td>-.18</td>
</tr>
<tr>
<td>F4</td>
<td>.03</td>
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</tr>
<tr>
<td>F3</td>
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<td>-.19</td>
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<tr>
<td>P3</td>
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<td>-.27</td>
</tr>
<tr>
<td>NREM 3</td>
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<td></td>
</tr>
<tr>
<td>F4</td>
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<td>-.16</td>
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</tr>
<tr>
<td>P3</td>
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<td>-.37b</td>
</tr>
<tr>
<td>NREM 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F4</td>
<td>.06</td>
<td>-.37a</td>
</tr>
<tr>
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<td>.02</td>
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<tr>
<td>P3</td>
<td>.12</td>
<td>-.31a</td>
</tr>
<tr>
<td>P3</td>
<td>.04</td>
<td>-.25</td>
</tr>
</tbody>
</table>

The significant correlations are marked as follows:

* \(p < 0.05\)

** \(p < 0.01\)

*** \(p < 0.001\)

Fig. 3. The duration of slow and fast sleep spindles as a function of sleep cycle (NREM period) and cortical region. The overall interaction between cycles, topography and spindle type is not significant. The significant post hoc Tukey HSD tests are marked as follows: * \(p < 0.05\), ** \(p < 0.01\), *** \(p < 0.001\). Note that the overall difference between the durations of slow and fast sleep spindles \((s < f)\) is marked on the figure. Vertical bars denote 0.95 confidence intervals.

Fig. 4 for details). In contrast with spindle density and duration no significant correlations are marked as follows: * \(p < 0.05\), ** \(p < 0.01\), *** \(p < 0.001\). Note that the overall difference between the amplitudes of slow and fast sleep spindles \((s < f)\) is marked on the figure. Vertical bars denote 0.95 confidence intervals.
significant correlations between sleep spindle amplitudes and age emerged (Table 2).

3.3. Comparison of automatic spindle detection with visual scoring

In total 2140 sleep spindles were identified by visual scoring. This value refers to both channels. Automatic detection identified 4780 sleep spindles. 92.9 percent of the visually detected spindles were matched by the IAM and 7.1 percent were not. In contrast 41.59 percent of sleep spindles detected by the IAM were matched by visual scoring, while 58.41 percent of IAM spindles were not.

Additionally, we report the overlap of visually and IAM-detected spindle periods. Visually detected spindles covered 7.36 and 11.7 percent of the whole scored EEG periods in channels F4 and P4, respectively. The corresponding values for IAM were 24.55 and 32.13. The 83.78 and 87.47 percent of visually detected spindle periods were congruous with the IAM spindle periods in channels F4 and P4, respectively. In contrast only 39.83 and 56.12 percent of IAM spindle periods were congruous with visual ones.

In order to further improve the direct comparison of our automatic and visual scoring of sleep spindling we correlated the numbers and densities (spindles/min) of IAM and visual spindles. This was performed on the merged dataset of EEG derivations F4 and P4. Both the number and the density of IAM spindles correlated positively with the number and the density of visually scored spindles ($r = .45$; $p < .024$ and $r = .41$; $p = .044$ for number and density, respectively).

In our second hypothesis we claimed that the spectral content of the sleep EEG segments covered by the IAM of sleep spindle analysis mirrors the individual-specific shape of the average spectra with increased values at the peaks reflecting sleep spindles. The parallelism between the average amplitude spectra of the whole analyzed segment (AVG), the spectra of automatically and visually detected spindles (A&VD), exclusively automatically detected (EAD) spindles, exclusively visually detected (EVD) spindles and segments with no detections (ND) was tested by calculating Pearson correlation coefficients in the frequency range of 9–16 Hz (for an example of different spectra see Fig. 6). At the EEG derivation F4 the correlations between A&VD and AVG ($r = .87$) as well as between EAD and AVG ($r = .95$) amplitude spectra were significantly higher than the correlations between EVD and AVG ($r = .58$; $t = 2.89$, $p = .0145$ and $t = 3.46$, $p = .0053$, respectively) or ND and AVG ($r = .48$; $t = 2.68$, $p = .021$ and $t = 3.83$, $p = .0027$, respectively) spectra (Fig. 7). Similar results emerged at derivation P4. Here the mean correlation of A&VD and EAD with AVG were .80 and .95, respectively, which significantly exceeded the correlation of EVD ($r = .45$; $t = 2.39$, $p = .0171$ and $t = 5.24$, $p = .0002$) and ND ($r = .38$; $t = 2.34$, $p = .039$ and $t = 4.27$, $p = .0013$, see also Fig. 7) with AVG. In addition the correlation between EAD spectra and AVG spectra was higher than the correlation between A&VD and AVG in derivation F4 ($t = 2.40$, $p = .035$).

Given the above mentioned high similarity of A&VD and EAD spectra with AVG spectra one might wonder if A&VD and EAD contain some specific elements in comparison with AVG. To reject the null hypothesis (A&VD and EAD did not differ from AVG) we compared the integrated amplitude spectra of A&VD, EAD, EVD, ND and AVG in the individual-specific spindle ranges (slow− fast) by repeated measures ANOVA with the factors segment (A&VD, EAD, EVD, ND, AVG) and cycle (1–4).
variables of sleep spindle spectra (Werth et al., 1997; De Gennaro et al., 2005; Buckelmüller et al., 2006). In our concept this should be the basis and at the same time the final test of sleep spindle analysis. An accurate automatic method of sleep spindle quantification should recognize spindles which are characterized by the individual-specific features observed in the EEG spectra. The visual detection of spindles is just a method of catching the typical and well-formed spindles, which – according to our present results – may contribute to the individual-specific spectral peaks, but represent just the cube of the iceberg.

The IAM was based on this theoretical background and the procedure detected much more sleep spindles than previous methods (see Fig. 2). It is important to note however, that slow- and fast sleep spindle densities are not additive because a given spindle sequence might contain both slow- and fast frequency components. A merge of IAM slow- and fast sleep spindle detections in the subsample used for the comparison of IAM with the visual detection resulted in an average spindle density of 11 spindles/min/derivations. Given this high value it is important to show that the visually non-detected IAM spindles (EAD) share the individual-specific spectral shape exceeding the average in the individual-specific spindle ranges. These statements were supported by the exceptionally high and significant correlations between EAD spectra and AVG spectra (see Figs. 6 and 7) and the difference between integrated spindle spectra of EAD and AVG (Fig. 8). EVD spectra were characterized by much lower similarity with AVG than the EAD spectra (Fig. 7). That means that those 7.1 percent of visually detected sleep spindles which were not covered by IAM (EVD) are characterized by

Fig. 6. Exemplary spectral profiles of matching and mismatching automatic and visual sleep spindle detections. A&VD, automatically and visually detected events; EAD, exclusively automatically detected events; EVD, exclusively visually detected events; ND, no detections; AVG, average. Note the striking similarity between the spectra of A&VD, EAD and AVG as well as the dissimilarity of EVD and ND. Moreover, both A&VD and EAD reliably reproduce the individual-specific spectral peaks, and exceed the AVG in the individual-specific spectral content. Difference emerges only in the amount of individual-specific spectral content: visual detection only catches the extremities of these spectral features (the cube of the iceberg), while automatic detection is more sensitive to the hidden and less emphatic features.

Fig. 7. Pearson correlation coefficients (Pearson r) expressing the parallelism of 9–16 Hz amplitude spectra of different EEG segments with the average amplitude spectra of all segments. A&VD, automatic and visual detections; EAD, exclusively automatic detections; EVD, exclusively visual detections; ND, no detections; AVG, average. The significant dependent sample t-tests are marked as follows: *p < .05, **p < .01, ***p < .001.

EVD, ND, and AVG) and topography (F4, P4). This resulted in a significant main effect of segment ($F = 24.99; d.f. = 4, 44; p < .000001$), without a significant effect of topography ($F = .93; d.f. = 1, 11; p = .35$) or interaction between segment and topography ($F = 1.51; d.f. = 4, 44; p = .21$). Given the lack of the effect of topography post hoc Tukey HSD tests were used for testing the segment effects only (with the collapsed data of derivations F4 and P4). Individual-specific spindle spectra of A&VD, EAD and EVD were significantly higher than the spindle spectra of ND and AVG (Fig. 8).

4. Discussion

The accurate analysis of sleep spindling has remarkable practical and theoretical importance. This is underlined by the reported cognitive (Bódizs et al., 2005, 2008; Clemens et al., 2005, 2006; Schabus et al., 2007, 2008; Fogel et al., 2007) and clinical neuropsychiatric (Donnet et al., 1992; Ferrarelli et al., 2007; Petit et al., 2004; Limoges et al., 2005; Gürses et al., 2005) correlates of different sleep spindle measures. Given the high interindividual variability of sleep spindle spectra (Werth et al., 1997; De Gennaro et al., 2005; Buckelmüller et al., 2006) we aimed to take into consideration this variability and to individually adjust the frequency and amplitude criteria of spindle analysis to the individual-specific features. This was done in order to avoid the splitting of individual spectral peaks by using ad hoc frequency criteria. In a previous publication (Bódizs et al., 2005) we developed the first version of our method in order to assess the relationship between sleep spindling and general mental ability. Though this first study supported the significance of separating slow- and fast sleep spindles, yet it also suffered from some methodological drawbacks. These were the ad hoc amplitude correction of the filtered signal and the lack of information on the duration and the amplitude of sleep spindles, which were shown to be important when investigating cognitive correlates (Schabus et al., 2007). In our present report we worked out these shortcomings by using an alternate method of bandpass-filtering and envelope detection. Moreover, we provided an extensive characterization of the output of the IAM by analyzing sleep cycle and age effects, topographical differences and a comparison with visual sleep spindle scoring. Our approach was based on the notion of the individual fingerprints in sleep EEG spectra (De Gennaro et al., 2005; Buckelmüller et al., 2006). In our concept this should be the basis and at the same time the final test of sleep spindle analysis. An accurate automatic method of sleep spindle quantification should recognize spindles which are characterized by the individual-specific features observed in the EEG spectra. The visual detection of spindles is just a method of catching the typical and well-formed spindles, which – according to our present results – may contribute to the individual-specific spectral peaks, but represent just the cube of the iceberg.

Fig. 8. Integrated individual-specific spindle spectra (slow + fast) in different EEG segments. A&VD, automatic and visual detections; EAD, exclusively automatic detections; EVD, exclusively visual detections; ND, no detections; AVG, average.
a non-specific spectral content, which does not resemble the AVG of the individual. Whether these spindles should be measured by some additional improved methodology or discarded in cognitive and clinical studies is a question which merits further attention.

The correlations between the numbers and densities of visually detected and IAM spindles were positive and significant, but relatively low. Thus the individual differences in the level of sleep spindling might be predicted by both the IAM and the visual detection, but the similarity of these predictions is only of about 20%. In our opinion the positive and significant correlations suggest the common backgrounds of the visually detected and the IAM spindles, but there might be an imperfect association between the variance in the inclusiveness of the two methods. Reasons for this imperfect association might be related to the differences between the features potentially influencing human pattern recognition and automatic detection based on the IAM. The former could be influenced by the presence of non-spindle frequency components or by the shape of the analyzed signal, while the latter is sensitive to the individual-specific spectral content only. Thus the inclusiveness of visual spindle detection might be more deeply influenced by factors like the shape of the actual spindle waveform or the presence of frequency components outside of the usual sigma range, while the IAM is practically unaffected by these intuitive features. However, one should ascertain, that we miss unequivocal data regarding the practical relevance of non-sigma frequency components embedded in the spindle sequences or of the shape of spindles. The IAM is based on the fingerprint theory of sleep EEG (De Gennaro et al., 2005; Buckelmüller et al., 2006) which considers the average spectra only and is not related to the above mentioned, visually striking spindle features.

The IAM of sleep spindle analysis resulted in slow- and fast sleep spindle detections which were preponderant in frontal and parietal derivations, respectively (Fig. 2). The similar, but less accentuated topographical patterns of spindle durations and spindle amplitudes were observed. Here we reported relatively increased levels of frontal and parietal slow spindling and fast spindling, respectively, but differences between the two types of spindling in the same topographic area did not follow the rule (Figs. 3 and 4). There is a large number of evidence underscoring the anterior predominance of slow and posterior predominance of fast sleep spindle measures (Gibbs and Gibbs, 1950; Zeithofer et al., 1997; Werth et al., 1997; Anderer et al., 2001). Our current results cohere with these data providing an enrichment of the existing data with the observation that it is spindle occurrence (density) which is the main source of the topographical difference, with spindle duration and spindle amplitude contributing much less to this effect.

As we did not found a significant effect for the sleep cycles in our results the IAM did not unequivocally reproduced the sleep-cycle-dependent decline in slow spindle frequency activity and the concomitant increase in fast sleep spindle frequency activity (Werth et al., 1997). Although, we reported significant differences between the first and the third or the first and the fourth sleep cycles in spindle density and duration (Figs. 2 and 3), these results cannot be considered vicarious with the hypothesized main effects or interaction effects of cycles with other factors. The only well established sleep cycle effect is the sleep cycle-dependent decrease in frontal sleep spindle amplitude (Fig. 5) which might cohere with the sleep-cycle-dependent decline in slow spindle frequency activity (Werth et al., 1997).

The herein reported effect of age on sleep spindling suggests that the fast but not the slow sleep spindle densities and durations measured in the third and in the fourth sleep cycle decrease with age. As the frequency of IAM fast spindles was 12.5–14 Hz in general (Table 1), our above results cohere with the finding of Landolt et al. (1996) who reported an age-dependent decrease in sleep spindle frequency activity, which is particularly enhanced in the third and in the fourth sleep cycle and restricted to the 12.25–14 Hz band.

In our data we also observed a left hemispheric predominance of fast sleep spindle density and duration, which – at least to our knowledge – was never reported in the literature. However, a similar pattern of hemispheric asymmetry is known to be characteristic for sleep-deprivation-induced EEG delta enhancement in humans. The latter was interpreted as a case of local sleep regulation reflecting the functional asymmetry between the dominant and non-dominant hemisphere (Achermann et al., 2001). Given the shared mechanisms of spindle and delta production (Steriade, 2003) it is reasonable to assume a similar role for spindle asymmetry, which could be the reflection of local corticothalamic functionality (Bódizs et al., 2008).

It is obvious that the IAM detects a relatively high number and long periods of wave episodes which do not possess the typical spindle-like shape. No other current method of spindle analysis would term these episodes as spindles. As this particularly high number and long periods of IAM spindle detections which were not considered spindles during the visual inspection could be interpreted as a very high percent of false positives we have to be cautious in interpreting the present results. A powerful method should be sensitive and accurate at the same time. Thus the possibility exists that our method is just inadequate for sleep spindle analysis. This statement is true if we consider visual inspection as a final test of validity. But is visual inspection a reliable method? We think that its reliability is based on the consensus theory of truth. Bare consensus may serve the function of a reference point for the discussion of alternative theories. Such alternative theories may start out from new empirical findings. Such findings might be the two types of sleep spindles (Schabus et al., 2007) and the genetic basis of the individual fingerprints of NREM sleep EEG spectra in the spindle range (De Gennaro et al., 2008). Indeed visual sleep spindle scoring was never proved to be reliable in the differential analysis of slow- and fast sleep spindles or in the unraveling of the individual fingerprints of sleep EEG spectra. However, one should consider that the IAM spindles contribute to the peaks in the spectrum, which are regarded as the fingerprints of sleep–EEG and spectral measures of spindles. The classic measures of spindle frequency activity derived from spectral power of the spindle frequency range are even more inclusive than IAM. The classic spectral measures cover not only the spectral peaks, but also the pink noise contribution of no-spindle intervals as well as the power spectra around and between the peaks. In our consideration these approaches are overly inclusive while the classical spindle detection methods which are tight to visual detection too exclusive. Moreover, neither is sensitive to the individual differences in frequency, forcing the EEG signal into the Procust bed of some predefined frequency limits.

Acknowledgements

This work was supported by the National Office for Research and Technology (NKFP-18/020/04) and the National Research Fund (OTKA TS-049785 and OTKA-48927). The first author is supported by the János Bolyai Research Fellowship of the Hungarian Academy of Sciences.

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