Spindle Density in Sleep of Normal Subjects

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Summary: We studied sleep spindle activity in ten normal young subjects, five males and five females. The subjects were recorded on magnetic tapes according to standard procedures, and the tapes were scored automatically by a system described previously. Spindle activity was analyzed on the C4-T4 lead of electroencephalogram, using a bandpass filter and discrimination of the integrated output of the filter. Results indicated that spindle density was the same in stages 2, 3, and 4, and was much lower in rapid eye movement (REM) sleep. In stage 2, K potentials were less abundant than spindles and exhibited a much smaller individual variability. Whereas spindle density was very stable across two nights in the same subject, there was wide variation between subjects. In addition, there was a sex difference; female subjects presented more than twice as many spindles as males. The density of spindles during non-REM sleep was constant throughout the recording and did not show any cyclic variation. Key Words: Sigma sleep spindles—Spindle density—Human normal subjects—Sleep stages—Individual differences—Sex differences.

Before the introduction of the computer in sleep analysis, it was generally accepted that spindles were essentially present in stage 2, much less common in stage 3, and absent in stage 4 and in rapid eye movement (REM) sleep. They are still considered to be one of the key criteria for scoring stage 2. However, more recent studies based on computer evaluation of sleep recordings have produced a different picture. Dumermuth et al. (1972), using spectral analysis, showed that a distinct peak between 13 and 15 Hz was present in all stages of non-REM (NREM) sleep and that a small peak in this same frequency also was often visible in REM sleep. Electronic devices giving a spindle count have indicated a somewhat lower overall density of spindles in stages 3 and 4 than in stage 2 (Silverstein and Levy, 1976; Di Perri et al., 1977). Other factors affecting the occurrence of spindles have been considered: age (Smith et al., 1979), rank order of the night (Di Perri et al., 1977), distribution over the scalp (Dumermuth et al., 1972; Di Perri et al., 1977),
drug effects (Gaillard et al., 1973; Johnson et al., 1976), and pathology (Cherpillod et al., 1965; Feinberg et al., 1967; Johnson et al., 1970). Although it is generally admitted that spindle density is a very stable individual characteristic, discrepancies exist in the literature regarding the magnitude of the effect of the different factors listed above. In addition, and rather surprisingly, the influence of sex has not been systematically investigated previously.

The purpose of this paper is to describe spindle activity in the sleep of normal male and female subjects by investigating densities in the different sleep stages and the evolution of this density during regular nocturnal sleep.

EXPERIMENTAL PROCEDURES

Subjects

Ten subjects, five males and five females, were recorded for two nights each after one night of habituation in the laboratory. They were all in good health, as assessed by clinical interview, and did not complain of any sleep disturbance. Mean age was 31 ± 11 years (males) and 35 ± 12 years (females). They did not take any drugs and were asked to avoid alcohol and naps during the recording session.

Methods

The subjects reported to the laboratory about 1 hr before their usual bedtime and slept undisturbed until their desired rising time, so that their usual sleep schedule was not modified acutely. Electrodes were attached, as described previously, for electroencephalogram (EEG) monitoring: F4-CZ, C4-T4, and PZ-02 (Gaillard and Hovaguimian, 1976); electro-oculogram (EOG) (external angle of each eye); electromyogram (EMG) (two submental electrodes 5 cm apart); and electrocardiogram (ECG) (both forearms). All recordings were made using a standard EEG machine and a tape recorder.

Sleep scoring was done off-line with an automatic system described previously (Gaillard and Tissot, 1973). The software of this system was modified recently, to enhance the reliability of staging and to give more detailed information about sleep stage characteristics (Gaillard, 1978). In addition to the usual listing containing the sequence of minute-by-minute sleep stages, this new program produces on request a file on disk of the minute-by-minute count of additional parameters such as the activity in all EEG frequency bands, K potentials, and peripheral parameters. This information was analyzed subsequently by a set of programs for statistical estimation and for the study of the evolution of these parameters throughout the sleep period.

Spindle activity was analyzed on the C4-T4 EEG track. The location of the electrodes on the scalp selected for this analysis was not critical as it has been shown that there is a significant coherence between anterior and posterior derivations in the sigma band (Dumermuth et al., 1972). The signal recorded on the tape was passed to an active analog filter of the butterworth type, with a 6 db per octave attenuation. The center frequency of this filter was 14.7 Hz, and its
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bandwidth (3-dB attenuation) was 11.6–17.2 Hz. The output of the filter was rectified, integrated, and threshold-discriminated. The output of the comparator was sensed every 160 msec, to give a value of one if the output was below the threshold and of zero if it was above (the system works in negative logic). The criterion for acceptance of a spindle was an EEG activity within the bandpass of the filter during 480 msec or more and greater or equal to 3.75 $\mu$V of amplitude.

The statistical analysis included the calculation of classical estimators such as Student's $t$-test, Pearson's correlation coefficient, and two-way analyses of variance. The level of significance was set at 0.05.

RESULTS

Table 1 shows the density of spindle activity in the different sleep stages. This density was calculated by dividing the total number of responses in each sleep stage by the total number of minutes of these stages during the sleep period. It was not calculated for stage 1 because this stage was too short to give reliable results in this sample of normal subjects. The density of spindle activity was approximately the same for the three main stages of NREM sleep, and there was no decrease from stage 2 to stage 4. Although much lower, density in REM sleep was not negligible. In our automatic scoring system, the absence of spindles is one of the criteria for the recognition of this state, but more weight is given to two other criteria: the abolition of muscle tone and the occurrence of rapid eye movements. There are circumstances in which one or two spindles per minute are allowed in REM sleep, particularly in the absence of K potentials, if the other two criteria are met.

K potentials are another key component of stage 2 and traditionally have been described as often closely associated with spindles. However, the number of K potentials was much lower than the number of spindle responses in stage 2. In addition, these two components showed wide differences in variability.

A two-way analysis of variance (subjects/stages) indicated no significant differences between the three stages (i.e., 2, 3, and 4) of NREM sleep but a highly

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K potentials, stage 2</td>
<td>3.10</td>
<td>0.60</td>
<td>19</td>
</tr>
<tr>
<td>Spindles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 2</td>
<td>5.21</td>
<td>3.70</td>
<td>71</td>
</tr>
<tr>
<td>Stage 3</td>
<td>6.46</td>
<td>5.81</td>
<td>90</td>
</tr>
<tr>
<td>Stage 4</td>
<td>6.82</td>
<td>6.60</td>
<td>97</td>
</tr>
<tr>
<td>REM sleep</td>
<td>0.87</td>
<td>1.74</td>
<td>200</td>
</tr>
</tbody>
</table>

Reduction statistics of the mean number per minute of K potentials in stage 2, and of spindle activity in all sleep stages. These statistics were not computed for stage 1, which was too short in these normal subjects. SD, standard deviation; CV(%), coefficient of variation.
significant difference between these stages and REM sleep ($F = 12.17, df = 3, 27, p < 0.01$). As listed in Table 2, the density of spindle activity in female subjects was more than twice as high as in male subjects. However, large interindividual variability prevented the differences from being significant in stages 3 and 4. In order to evaluate this variability more precisely, and contrast it to night-to-night variation, another analysis of variance with the factors subjects/nights was computed. When all ten subjects were considered together, the differences among them were highly significant in all stages of NREM sleep ($F = 5.7, 48.41, and 44.5$ for stages 2, 3, and 4, respectively; $df = 9.9; p < 0.01$). However, in REM sleep, there was no significant subject effect ($F = 2.32; df = 9.9; p > 0.05$). There was no difference between nights, except for stage 3 ($F = 6.35; df = 1.9; p < 0.05$). This difference occurred in female subjects only, who showed (probably by chance) a systematic decrease in the density of spindle activity during the second recorded night. In addition, for all sleep stages, there was a highly significant correlation between the two nights for each subject ($p < 0.01$). The interindividual variability was not due only to male/female difference; the analysis of both sexes separately confirmed a significant subject effect in all stages of NREM sleep ($F = 13.87, 16.98, and 7.96; df = 4, 4; p < 0.05$ for males; $F = 26.45, 48.6, and 70.41; df = 4, 4; p < 0.01$ for females).

The absolute number of spindle responses rose sharply shortly after sleep onset, then declined progressively toward the end of the night, with some fluctuations due to the cyclic recurrence of REM sleep. If the proportion of sleep stages $2 + 3 + 4$ is calculated with respect to clock time, a similar evolution appears. The density of spindle activity, which is the ratio of these two time functions, did not show any more striking variations during the sleep period (Fig. 1). The small fluctuations still visible do not suggest any cyclic component; thus the density of spindle activity can be considered as constant across the stages of NREM sleep throughout the night.

**DISCUSSION**

There is no ambiguity in the study of the density of sigma-spindles in the stages of NREM sleep, since in our automatic system, as well as in the scoring rules given by Rechtschaffen and Kales (1968), the discrimination between stages 2, 3, 4, and REM sleep is straightforward.

**TABLE 2. Mean number of spindle responses per minute during each sleep stage in five male and five female subjects**

<table>
<thead>
<tr>
<th>Sleep stage</th>
<th>Males</th>
<th>Females</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$t \quad p$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.91 ± 1.81</td>
<td>7.51 ± 3.78</td>
<td>2.45 $&lt;$ 0.05</td>
</tr>
<tr>
<td>3</td>
<td>3.09 ± 2.75</td>
<td>9.83 ± 6.31</td>
<td>2.18 $&lt;$ 0.01</td>
</tr>
<tr>
<td>4</td>
<td>3.20 ± 3.17</td>
<td>10.45 ± 7.43</td>
<td>2.00 $&lt;$ 0.01</td>
</tr>
<tr>
<td>REM</td>
<td>0.09 ± 0.05</td>
<td>1.65 ± 2.30</td>
<td>1.52 ns</td>
</tr>
</tbody>
</table>
and 4 is based on the abundance of slow waves. There may be some ambiguity, however, in the case of REM sleep, because the absence of spindles is one of the accepted criteria for identification of this stage. It may be true that there should be no spindles in REM sleep. Nevertheless, it is well known that this stage is not always perfectly stable, inasmuch as physiological state changes evolve more smoothly than our clear-cut categories imply. Spindles do not necessarily occur only at the beginning and end of REM sleep, i.e., during the transition period to or from stage 2, but may occur in the middle of a REM episode. In sleep scoring, it is not reasonable to shift from "REM sleep" to "stage 2" for a few seconds as soon as a spindle appears on the tracing. The good agreement in the amount of REM sleep considered as normal in different laboratories clearly indicates that most sleep scorers, human as well as electronic, adhere to the same rules (Gaillard and Tissot, 1976). From a physiological point of view, these spindles probably represent small intrusions of some of the mechanisms of stage 2 in REM sleep. They are
more frequent in the first REM episode of the night. In this respect, our data are in close agreement with the results of spectral analysis, showing a peak in the sigma band in REM sleep (Dumermuth et al., 1972).

In their analysis of sleep EEG waveforms, Smith et al. (1979) found a density of spindles in stage 2 very close to our results. They used a different electronic system, consisting of a filter with a 12 db per octave attenuation outside the bandpass, combined with a zero-crossing analysis of the output of the filter. Their experiment showed that this combination is very efficient in eliminating false alarms that may occur, particularly in waking when some muscular activity persists. The spindle density reported by Di Perri et al. (1977) is also very close to our data.

The human sleep scorer usually considers spindles to be rare in stage 4; however, electronic devices identify an appreciable number of spindles in this stage. These discrepancies probably are due to the fact that a spindle superimposed on the flank of a slow wave is not easily recognized as such by the visual observer, whereas a filter discriminates the two frequencies. Silverstein and Levy (1976), using the spindle detector of Smith et al. (1975), described a markedly lower spindle density in stages 3 and 4 than in stage 2 in normal males. This is at variance with our data; we found a similar spindle density in all three stages of NREM sleep. The explanation may reside in slight differences in the definition of "spindle" for the system. Our filter and Smith's have almost the same bandwidth; however, the attenuation is different (12 dB per octave for the Smith filter, 6 dB per octave for ours). In the latter system, a spindle is counted if 87.5% of the frequencies within a period of 420 msec and with an amplitude higher than 5 $\mu$V falls in the bandpass of the filter. In our system the corresponding time and amplitude criteria are 480 msec and 3.75 $\mu$V. Our experience shows that the attenuation of the filter is not extremely critical to its performance, except that a 12 db per octave filter tends to produce more ringing. It is possible that the different characteristics of the two systems partly account for the fact that the results of Silverstein and Levy (1976) and ours are similar for spindle count in stage 2 but different for stages 3 and 4. In addition, they used male subjects only.

The highly stable individual characteristics of spindles that they report, in contrast to wide intersubject variation, was also confirmed by our results. The marked differences in density and variability of spindles with respect to K potentials may indicate that these two waveforms are not always as closely associated as is sometimes believed. Moreover, K potentials do not seem to bear as much of an individual signature as do spindles.

The observation of a higher spindle density in female subjects has not been reported previously. No explanation can be proposed at present for this difference, but it contrasts to the close similarity of most other sleep parameters in both sexes for the age of the subjects studied here (Williams et al., 1974). An interesting hypothesis to examine is that this greater density could be related in some way to the slightly better stability of sleep in normal female humans.

In addition to their frequency, other features of spindles can be measured with suitable electronic devices, particularly their amplitude and average frequency (Principe and Smith, 1982). A detailed analysis of this sort would allow further exploration of the modification of these waveforms in pathological conditions.
The evolution of sigma spindles during sleep has been examined in two papers. Keane et al. (1977) described a decrease of spindle density during the night, but it is not completely clear whether the time base they used was NREM sleep time or clock time. In the latter case, the decline of sigma density may simply represent the increasing production of REM sleep toward the end of the night. Silverstein and Levy (1976) showed a good stability of spindles during stage 2 and a small decrease during stages 3 and 4 with the time of the night. We have not systematically examined the evolution of spindles in separate stages, but the overall results suggest a very constant spindle density throughout the night. The absence of cyclic variations is in good agreement with our previous observations that the generator of the ultradian REM-NREM sleep cycle probably is linked to the mechanisms of REM sleep (Gaillard, 1979).

REFERENCES
Principe JC and Smith JR. Spindle characteristics as a function of age. Sleep 1982, in press.

Sleep, Vol. 4, No. 4, 1981