Pupillographic Assessment of Sleepiness in Sleep-deprived Healthy Subjects

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Summary: Spontaneous pupillary behavior in darkness provides information about a subject's level of sleepiness. In the present work, pupil measurements in complete darkness and quiet have been recorded continuously over 11-minute periods with infrared video pupillography at 25 Hz. The data have been analyzed to yield three parameters describing pupil behavior: the power of diameter variation at frequencies below 0.8 Hz (slow changes in pupil size), the pupillary unrest index, and the average pupil size. To investigate the changes of these parameters in sleep deprivation, spontaneous pupillary behavior in darkness was recorded every 2 hours in 13 healthy subjects from 19:00 to 07:00 during forced wakefulness. On each occasion, comparative subjective sleepiness was assessed with a self-rating scale (Stanford Sleepiness Scale, SSS). The power of slow pupillary oscillations ($\leq$ 0.8 Hz) increased significantly and so did the values of SSS, while basic pupil diameter decreased significantly. Slow pupillary oscillations and SSS did not correlate well in general but high values of pupil parameters were always associated with high values in subjective rating. Our results demonstrate a strong relationship between ongoing sleep deprivation and typical changes in the frequency profiles of spontaneous pupillary oscillations and the tendency to instability in pupil size in normals. These findings suggest that the results of pupil data analysis permit an objective measurement of sleepiness.

Key words: Pupil; pupillary oscillations; sleep deprivation; sleep disorders; Stanford Sleepiness Scale

THERE IS STRONG DEMAND for a convenient and objective measurement of sleepiness in sleep investigation and sleep medicine. Such methodology would have applications of potential value in industrial medicine, clinical pharmacology, psychiatry and psychology.

Sleepiness-related changes in spontaneous pupillary behavior in darkness were first described by Lowenstein et al.[1] Lowenstein was the first to report so-called fatigue waves: slow oscillations in pupil size with high amplitudes. Yoss[2] found pupillography a useful tool in the diagnosis and therapy management of narcolepsy. Since then, pupillography as an objective test of vigilance has been used only sporadically.[3-8]

Vigilance effects on the pupil are presumed to be mediated by variations in supranuclear inhibition on the Edinger-Westphal nuclei in the midbrain. In order that pupillary behavior may truly reflect vigilance, however, it is crucial that the nuclei be deprived of complicating afferent inputs such as occur with light or sound stimuli. Complete darkness and quiet are therefore necessary for a pupillographic sleepiness test. While light-induced oscillations in steady light depend on light intensity,[9] their frequency being a linear function of this intensity, spontaneous pupillary oscillations in darkness reveal a typical vigilance-related behavior. Low-frequency components are dominant in persons with excessive daytime sleepiness. The amplitude of slow oscillations (< 0.5 Hz) can reach several millimeters. On the other hand, in an alert subject, pupil diameter is kept stable for a long time, oscillating with a maximal amplitude of 0.3 - 0.5 mm, and the power of frequencies below 0.8 Hz is usually poor.

Because sleep deprivation provokes sleepiness which increases during the hours of usual night sleep, the numer-

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ic parameters for pupillary instability are expected to increase in parallel. In order to establish pupillography as a standardized objective test of vigilance, it seems inevitable to analyze pupillograms quantitatively. The aim of this study was to introduce a method of mathematical analysis of pupillograms in darkness in order to answer two questions: (1) How do the numeric parameters describing spontaneous pupillary oscillations in darkness change in normals during sleep deprivation? (2) Can sleepiness be described quantitatively by parameters derived from pupillogram analysis?

METHODS

Subjects

We examined 13 healthy subjects between 21 and 28 years (5 females, 8 males). All had given their informed consent to the study, most of them being medical students. They were instructed to fulfill their usual sleep demand the night before the test, to get up between 08:00 and 09:00 and to avoid alcohol the whole day, caffeine and nicotine after 15:00. They came to the eye hospital at 19:00, and became acquainted with the pupil measurement and locations for the experiment during the first 2 hours before the main part of the experiment started around 21:00. All measurements were performed in a semi-darkened and quiet laboratory. Additional dark adaptation was not necessary because we were exclusively measuring spontaneous pupillary oscillations in darkness. The data of the first pupillary record during adaptation period to locations and experimental conditions did not enter into evaluation. Data analysis worked up results of five measurements; the last records were taken at 07:00-08:00. The volunteers had to stay awake and up all night long. Between the tests they were playing games, talking, reading or eating light meals. Infrared video pupillography was performed every 2 hours, followed by the filling in of the Stanford Sleepiness Scale.[10]

Experimental Conditions

The subject sat on a comfortable chair looking into the dark pupillographic device. The head was fixed by a chin rest and flexible goggles integrated into the aperture of the apparatus. Subjects were instructed to fixate a group of infrared light-emitting diodes which were used as the pupillographic light source and which glow dim red in the dark. The camera picture of the subject's eye was monitored on-line on one screen, while a second screen showed pupil diameter, refraction and changes of eye position graphically for consecutive periods of 20 seconds. Simultaneous monitoring of accommodation and pupil size permitted the detection and elimination of measurements showing near responses that also might cause a decrease of pupil size. In the present study, none of the records had to be excluded for that reason. During recording, the room was kept quiet and in complete darkness to avoid the influences mentioned above.

Infrared Video Pupillography

Spontaneous pupillary behavior in darkness was recorded over a period of 11 minutes by means of infrared video pupillography. The system consisted of an infrared sensitive videocamera connected with a frame grabber card in a 80386 personal computer, and image processing software for 25 Hz real time analysis. Details of the method of recording are described elsewhere.[11] The spatial resolution of the system was 0.1 mm. Eye position and refraction were recorded simultaneously by the same system.

Data Management and Parameters of Evaluation

Prior to further analysis, a mathematical artifact-rejection algorithm was applied to the raw data in order to remove blinks and high-frequency noise efficiently. The off-line analysis provided information about frequency profiles (Fast Fourier Transformation, FFT), changes of average pupil size with time, and the tendency of pupillary unrest. We used the FFT in order to analyze slow pupillary oscillations ≤ 0.8 Hz (slow changes in pupil size), reflecting the level of vigilance. At first the entire record of 11 minutes was divided into eight time segments of 81.92 seconds (=211 measurements) for this special analysis. The FFT including frequency bands from 0 - 0.1 Hz, 0.2 - 0.3 Hz up to 0.7 - 0.8 Hz was applied to each time segment. The FFT results were printed out for each individual measurement in both graphs and tables. The table presented the absolute and relative power of each single-frequency band per time segment, mean values of power for each frequency band (0.1 to 0.8 Hz) and time segment and mean values for the entire measurement.

(1) The mean value for the summed power values of all low frequencies ≤ 0.8 Hz of an 11-minute measurement was one of the parameters of evaluation.

(2) Pupillary unrest index (PUI) indicates the pupil´s instability during the measurement in darkness. The PUI is a measure of the slow movements of the pupillary margin during the measurement. A stable pupil diameter of an alert subject is associated to low values in PUI, while strong fluctuations in pupil size in a sleepy subject produce high values of PUI. Before calculating the PUI, low-pass filtering of the signal with a cutoff frequency of 1,56 Hz was applied. The mean of 16 data points was calculated, along with the difference to the mean of the following 16 data points. This procedure was applied to the entire data set, and all differences (mm) were summed up and calculated per minute. Therefore, the PUI for each measurement was

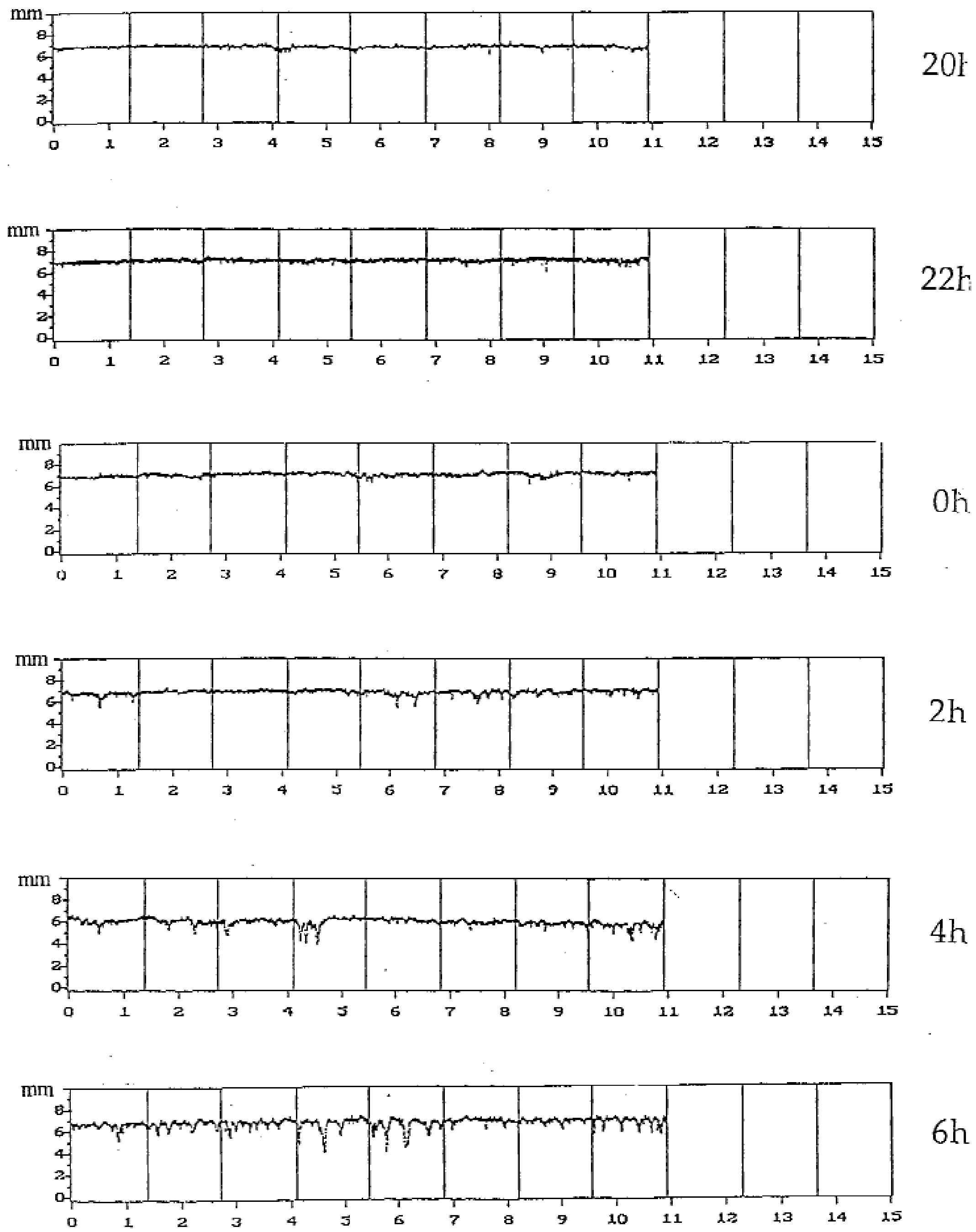

Figure 1.—Pupillographic records during the course of the sleep depriving experiment in one subject. At six different times during the night of forced wakefulness from 8 p.m. to 6 a.m pupil size (mm) is plotted against time (11 minutes). Instability of pupil diameter increases with hours of ongoing sleep deprivation.

given in mm/minute. This parameter has been suggested by Irene Loewenfeld (personal communication).

(3) The average pupillary diameter per time segment (82 seconds) and a mean for the entire record was calculated and plotted.

Statistics

Purpose of statistical analysis was to find out if there is an increase in power ≤ 0.8 Hz (increase in PUI, decrease in pupil size) during forced wakefulness. In order to avoid the effect that one single subject with strong increase or decrease might have a high influence on the results, we determined the linear line fit (as shown in Fig. 2) of each pupil parameter (PUI, power ≤ 0.8 Hz and average pupil size) and also of SSS-values for every subject in the course of the experiment. The slope was positive if there was an increase of a parameter during the experiment. To test the alternative hypothesis (ie, increase existing) against the

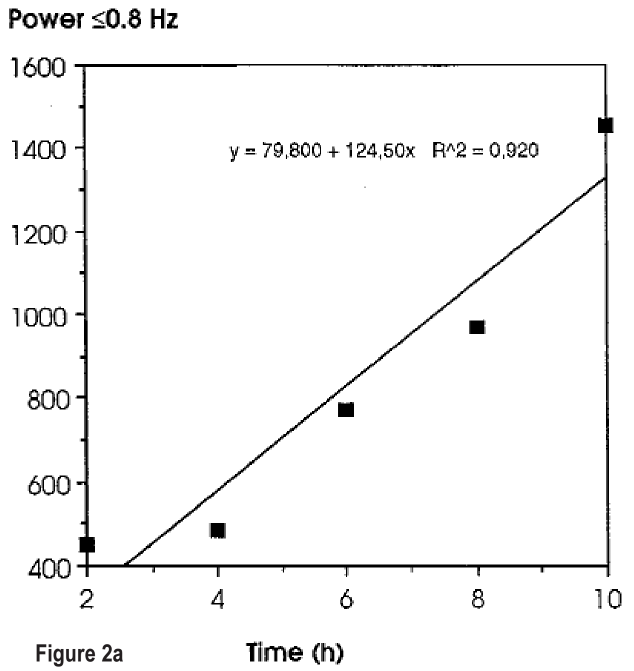

Figure 2a

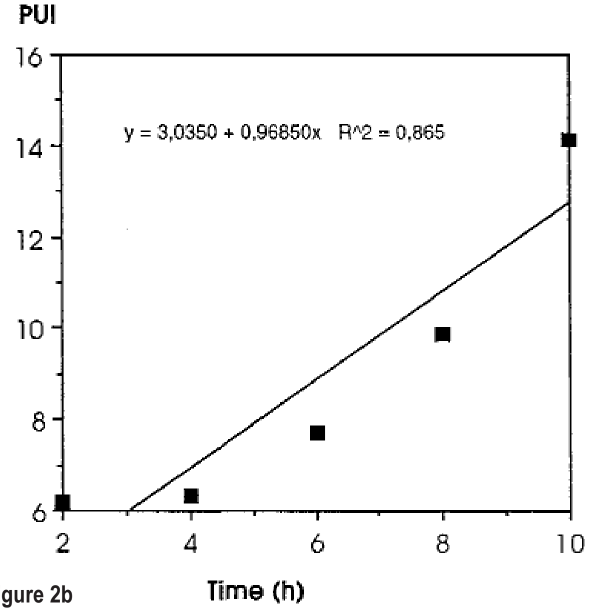

Figure 2b

Figure 2.—Power of frequencies ≤ 0.8 Hz (a) and PUI (b, pupillary unrest index) against time waking in the same subject as demonstrated in Fig. 1. There is an increase of the numeric parameters with ongoing sleep-deprivation. A linear line fit is shown for each parameter.

null hypothesis (ie, no progression with ongoing sleep deprivation), we expect that the mean of the slopes is significantly larger than 0. This procedure was done by the one-tailed one-sample t-test, as the values showed a normal distribution. Additionally, we applied ANOVA with repeated measurements to test the increase of power and PUI during forced wakefulness. With a polynomial contrast we got a test for constant, linear, quadratic, or cubic, etc., term of the development of the parameters against the time points. Furthermore, progression of sleepiness-related changes within each single measurement was evaluated by the Student t-test for paired samples.

The correlation between SSS values and pupil data for all subjects (n=13) was calculated for each time of measurement of the experiment (6 records). We applied the Spearman rank coefficient to indicate the degree of association between these variables.

RESULTS

Power ≤ 0.8 Hz and Pupillary Unrest Index (PUI)

Both numeric parameters for pupil size changes increased significantly (one-tailed one-sample t-test: power [t-value= 6.82, df=12, p<0,001], PUI [t-value=6.11, df=12, p<0,001]) during the night waking (Fig. 1-4). Figure 1 shows an example of changes in pupillary behavior between the different times of recording during the experiment in one subject, while the change of PUI and power of slow oscillations in this subject is demonstrated on Fig. 2. The difference in power and PUI between an alert and a sleepy measurement of one subject is demonstrated in Fig. 3, the numeric parameters (power and PUI) expressing rising activity of slow pupillary oscillations are higher in sleepiness.

Even though the interindividual variability of absolute values was high, the increase during the night was present in each individual (Fig. 4a and Fig. 4b). We applied post hoc testing to analyze whether the power and PUI increased not only linearly but also with the square of time and found significance for this relationship, too (ANOVA, repeated measurements, power [t-value of quadratic term of polynomial contrast = 2.54, df=12, p=0.025], PUI [t-value of quadratic term of polynomial contrast=2.87, df=12, p=0.014]).

The amount of increase of slow pupillary oscillations and PUI was different in different individuals, and showed up weakest in a student frequently jobbing as a night nurse, probably indicating an influence of sleep-wake habits on her pupillary behavior. The dynamics of pupillary parameter within each single session were progressive, the second half (minute 5.5 to 11) of the session showing higher values than the first half (minute 0 to 5.5). We compared the differences of the parameters' power of the first four time segments to those of the last four, and found these differences to be non-significant for the 22:00 and 04:00 records. All other times showed significantly higher values in the second half of session ([two-tailed t-test for paired samples, df = 12] 24:00: p = 0.019, t=-2.71, 02:00: p=0.023, t=-2.62, 06:00: p=0.014, t=-2.88) (Fig. 5).

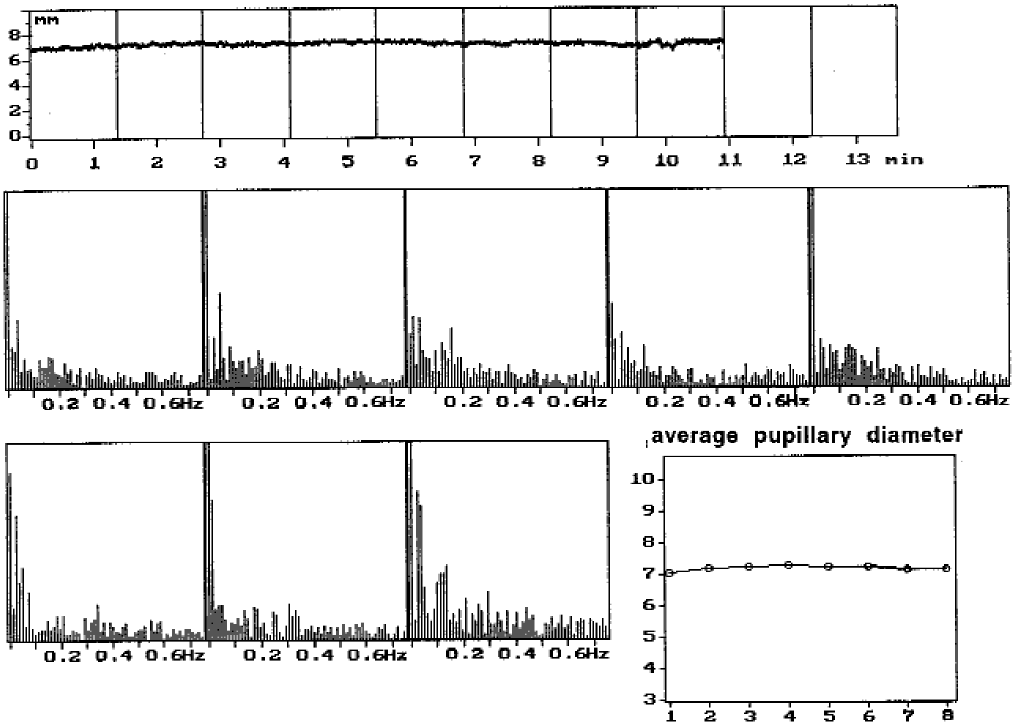

Figure 3a

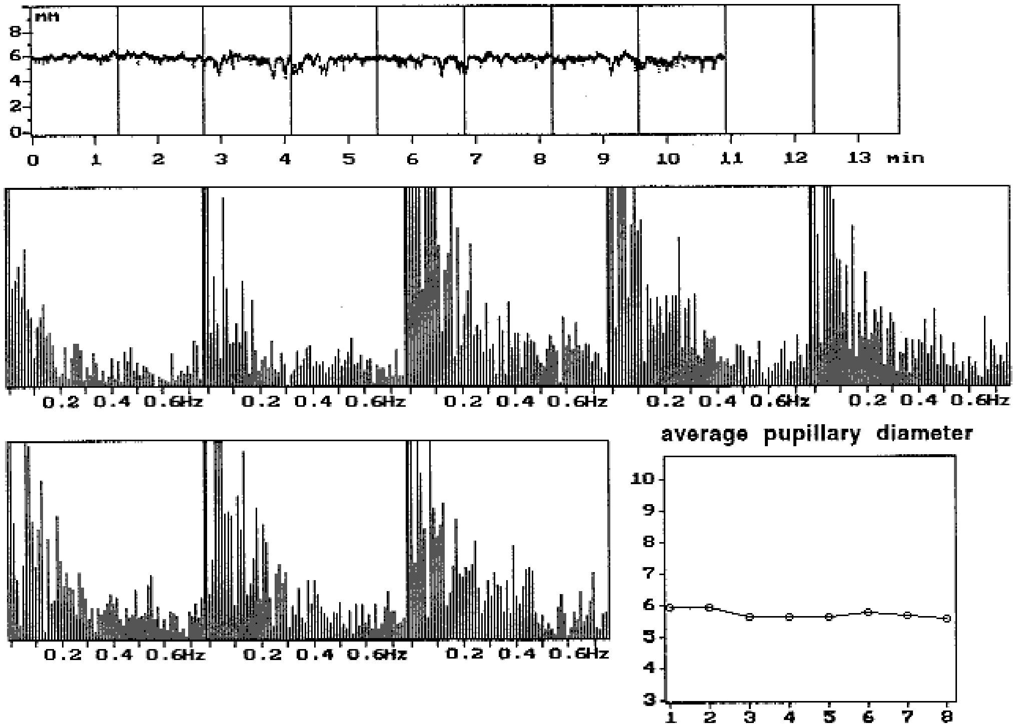

Figure 3b

Figure 3.—Example: Pupillograms and results of frequency analysis (FFT) of one subject in alertness (a) at the beginning and in sleepiness (b) at the end of the experiment. Eight windows show the FFT-results graphically. The small graph (bottom, right) presents changes in mean pupil size per time segment during a single measurement. Numeric results of measurement (a): power 826, PUI 4.11; measurement (b): power 2469, PUI 11.

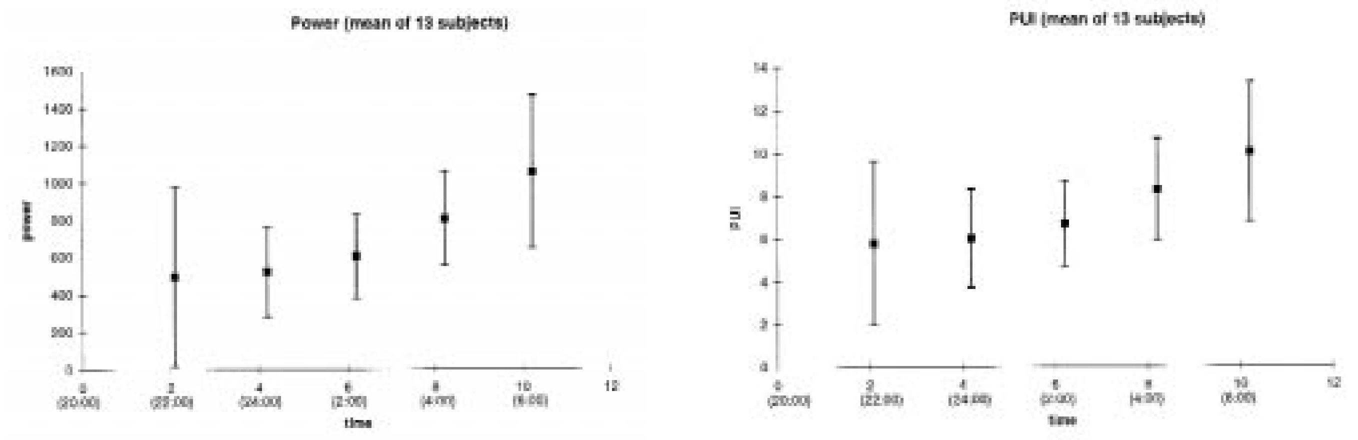

Figure 4a

Figure 4b

Figure 4.—Power ≤ 0.8 Hz (a) and PUI (b) against time waking, mean of 13 samples ± SD. Significant increase of both numeric parameters (ANOVA repeated measurements, p < 0.001) with the square of time.

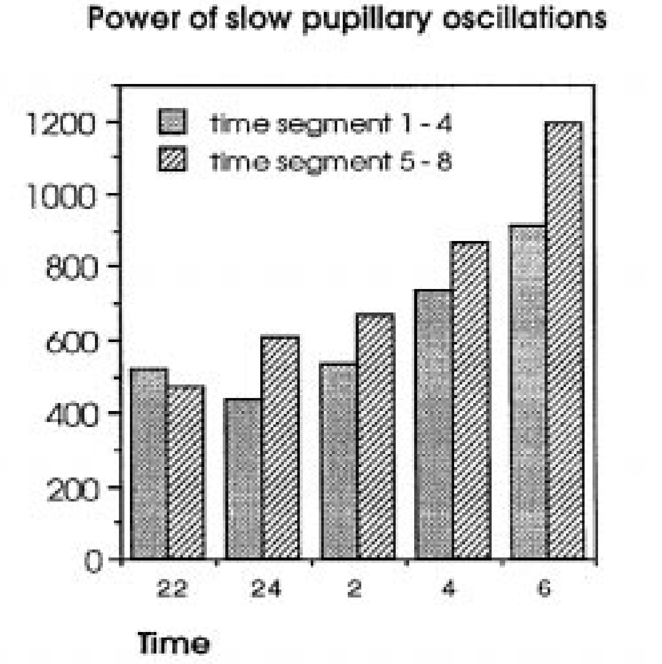

Figure 5.—Comparison between first half (time segment 1 - 4, dark, lower part of graph) and second half (time segment 5 - 8, brighter, upper part) of five measurements. In most instances, the second half shows higher numeric pupillary parameters expressing a decreasing level of vigilance during the measurement (significant at 24:00, 02:00, and 06:00).

Pupil size.—Pupils became significantly smaller under forced wakefulness [one-tailed one-sample t-test , t=2.26, df=12, p=0.022](Fig. 6).

Stanford sleepiness scale (SSS).—The subjective rating values increased significantly [one-tailed one-sample t-test, t=10.82, df=12, p< 0,001] with ongoing time of the experiment (Fig. 7). We applied a regression analysis to the pupil data related to SSS values at the different times of measurement and found no correlation. There was a high variability of subjective rating for low and mean power. But when a subject reached high values in pupil parameters the values in SSS always were high, too.

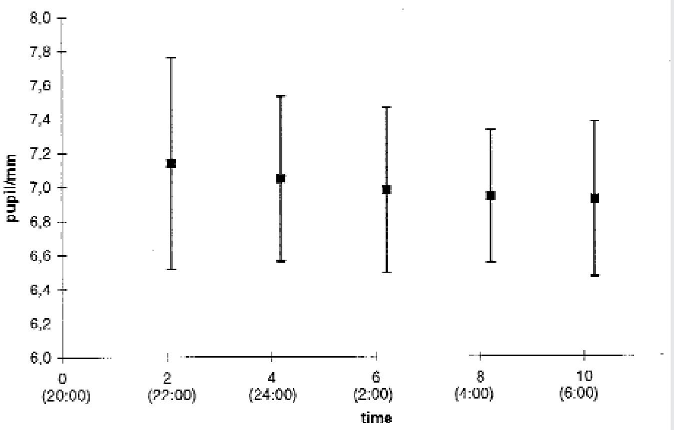

Figure 6.—Changes in average pupil size per measurement during sleep deprivation, mean of 13 samples ± SD. Pupil size decreases significantly with ongoing forced wakefulness (p = 0.022).

DISCUSSION

Our results confirm those of Lowenstein et al[1] and Yoss et al,[2,3] demonstrating a clear relationship between sleep deprivation and typical changes of spontaneous pupillary behavior in darkness. In our study the pupillograms were evaluated quantitatively. Pupillary low frequency oscillations increase clearly with the time of sleep deprivation, ie, they increase parallel to sleepiness. Therefore, numeric values obtained by pupillogram analysis are able to quantify

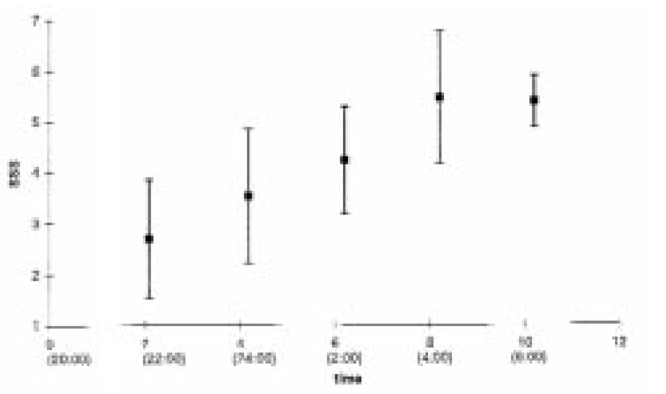

Figure 7.—Values of self-rating on Stanford Sleepiness Scale (mean of 13 samples ± SD) increase significantly during the sleep-deprivation experiment (p < 0.001).

sleepiness. The progression in pupil parameters in the time course was highly significant; PUI and power increased with the square of time. Simultaneously there was a significant decrease of pupil size with ongoing forced wakefulness. Absolute pupil parameters varied considerably between different individuals. In spite of showing similar significant progression with ongoing time, subjective rating of sleepiness did not correlate with the pupillary findings. However, high measures for pupillary instability were seen only in subjects feeling tired. The same poor correlation to subjective rating has been reported for the multiple sleep latency test (MSLT).[12] In both instances, we deal with a variety of influences (eg, motivation, self-perception) on subjective measures which have no effect on the basic level of central-nervous activity as measured by MSLT or pupillography.

Several authors have dealt with spontaneous pupillary behavior in darkness in the assessment of daytime sleepiness.[1,3,4,7,13] Although there are great methodological differences, all authors agree on the usefulness of pupillary signs in the detection of excessive daytime sleepiness.

All studies mentioned differ from ours in the time of recording pupil size, parameters of evaluation (changes in pupil size or changes in spontaneous oscillations), data management, or both.[5-7,14-16] Hertz et al[16] found a correspondence between pupillometric and MSLT findings in sleep-deprived healthy controls even when analyzing pupil size as briefly as 2 minutes after having recorded a number of pupillary light reactions. Schmidt found pupillography a helpful tool in the assessment of sleepiness correlating well with MSLT results,[8,15,17] by exclusively measuring basic pupil sizes every half-minute over a period of 10 minutes and evaluating steps between single measurements. Lichstein[6,7,14] investigated pupillary characteristics of insomniacs in different age groups, and found average pupil size in two studies to be significantly smaller than in normals, but the differences in magnitude were very subtle and in a further study they were statistically non-significant. In Lichstein´s studies, the difference in oscillations was non-significant, probably due to the fact that instead of a frequency analysis of oscillations, a mean count of oscillations per minute was considered.

Methods similar to ours in recording spontaneous pupillary oscillations in darkness, artifact management, and data analysis have been described by McLaren and Merritt[4,18] for clinical use in narcoleptic patients. As far as parameters of evaluation are concerned, McLaren compared subjective assessment of pupillograms to numeric parameters, and found the best correlation for a combination of results from Fourier Transformation plus parameters of miosis evaluation. He did not use parameters similar to the power ≤0.8 Hz or the pupil unrest index (PUI). Our study showed that these single parameters of the FFT and the PUI were sufficient to describe pupillary instability with the purpose of assessing sleepiness.

CONCLUSIONS

Compared to earlier studies dealing with patient groups suffering from hypersomnia or insomnia, the present study documents the positive correlation between ongoing sleep deprivation and spontaneous pupillary oscillations in darkness in sleep-deprived healthy subjects. It proved to be possible to quantify sleepiness objectively by analyzing the spontaneous pupillary oscillations in darkness with common mathematical methods. The power of low frequency oscillations and the Pupillary Unrest Index (PUI) increased significantly with the time of forced wakefulness.

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