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REGULAR ARTICLE

Association between pupillometric sleepiness measures and sleep latency derived by MSLT in clinically sleepy patients

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Abstract
Objectives The multiple sleep latency test (MSLT) has been employed extensively in clinical and research settings as a gold standard for objectively measuring sleepiness. In a general population or in a variety of work settings, however, a more convenient, rapidly administered measuring method is preferable. We examined the potential utility of pupillometry by comparing its objective measures, pupillary unrest index (PUI) and relative pupillary unrest index (RPUI), with MSLT-derived sleep latency (SL).
Methods The study cohort comprised 45 patients (39 males, 6 females, mean age 38.9 ± 11.3 years) referred to the Sleep Disorders Center for the two-nap SL test. SL was measured twice before noon, and pupillometric measurement was performed immediately before each SL test. Subjective sleepiness was measured by using the Epworth Sleepiness Scale (ESS).
Results The association between PUI and SL was significant and far closer than that between RPUI and SL. A significant difference was observed between the two groups, based on each subject's experience of drowsy driving accidents over the past 3 years in the PUI and RPUI, as well as in SL. The subjective sleepiness measure, ESS, did not relate to any other physiological sleepiness measures.
Conclusions In our study cohort, the pupillometric sleepiness measure, PUI, was significantly correlated with, and behaved in a manner equivalent to, MSLT-derived SL in clinically sleepy patients. However, several points remain to be carefully examined before applying pupillometry for screening sleepiness in a general population, or in occupational settings.

Keywords Pupillary unrest index · MSLT · Sleep latency · Traffic accident · Epworth Sleepiness Scale

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Introduction

In modern society, the negative impact of excessive sleepiness has emerged as a significant problem in terms of health, quality of life, and work performance. In order to be able to apply the best approach to deal with excessive sleepiness, a proper assessment of the condition is essential.

The multiple sleep latency test (MSLT) was developed in the late 1970s to obtain objective physiologically based information about sleep tendencies [1] and has been employed extensively as the gold standard for objectively measuring physiological sleepiness [2]. It assesses the mean number of minutes people take to fall asleep after being instructed to do so, while lying down in a quiet, dark environment with eyes closed during two to five 20-min daytime nap opportunities scheduled 2 h apart [3, 4]. The MSLT procedure is rather expensive, time-consuming, and somewhat annoying. It requires technical expertise, and application of the test is usually restricted to clinical and research settings. Consequently, for the screening of sleepiness in a large general population or in a variety of work settings, a more convenient, noninvasive, rapidly

administered, physiologically based, and reliable measuring method is preferable.

The phenomenon of sleepiness-induced oscillations of the pupil diameter has been labeled as pupillary unrest (PU) [5]. In the late 1990s, the pupillographic sleepiness test (PST) was developed using pupillometry for the quantitative assessment of PU [6, 7].

Spontaneous pupillary oscillation in darkness has been recorded by infrared video pupillography to quantify daytime sleepiness both in healthy subjects, such as sleep-deprived subjects [8], truck drivers [9], physicians following night duty [10], and neurology residents after night shift [11], and in patients with sleep disorders [7, 12, 13] or neurological diseases, such as Parkinson's disease [14] or multiple sclerosis [15]. These studies proved that PU is a valid and objective indicator of reduced arousal and showed pupillometry to be a useful tool for the screening and measurement of alertness whenever this outcome is of interest and for the monitoring of treatment for sleep disorders or in clinical trials. However, although the association between sleepiness measured by pupillography and MSLT has been addressed in many studies, the results to date have been inconclusive [12, 13, 16, 17] for many reasons, such as small sample size [13].

The objectives of our study were to: (1) establish whether sleepiness measured by pupillometry correlates with the MSLT-defined sleepiness in a sufficient number of clinically sleepy patients and (2) determine the similarity between the two methods in relation with subjective sleepiness and with behavioral sleepiness episodes, i.e., the experience of drowsy driving accidents.

Methods

The study was reviewed and approved by the Institutional Research Board of Aichi Medical University School of Medicine, and all participants provided written consent for their participation.

The study cohort comprised 45 outpatients aged <60 years (39 males, 6 females; mean age 38.9 ± 11.3 years) of the Sleep Disorders Center, Aichi Medical University Hospital, who had been referred to the Center between 13 December 2005 and 31 August 2008 for the two-nap SL test to clarify their excessive daytime sleepiness.

The final clinical diagnoses of these patients were sleep apnea syndrome (28 patients, 62.2 %), hypersomnia (10 patients, 22.2 %), narcolepsy (4 patients, 8.8 %), idiopathic hypersomnia (1 patient, 2.2 %), behaviorally induced insufficient sleep syndrome (1 patient, 2.2 %), and depression (1 patient, 2.2 %).

Protocol

Subjects were instructed to refrain from caffeine for at least 12 h prior to sleepiness assessment and asked to sleep for >6 h during the previous night. On the day of assessment, they were requested to wake up at least 90 min before the start of testing. SL was measured twice in a dark quiet room before noon. Pupillometric measurement was performed immediately before each SL test.

Pupillometry

Pupil diameters were continuously recorded with an electronic pupillometer (F2D system; AMTech, Weinheim, Germany) for 11-Ãmin. The F2D system consisted of a computer with installed software, a key board with track ball, a TFT-monitor, video goggles with infrared illumination, a printer, and a set of power supply. These components fit into two portable attaché cases.

Prior to measurement, subjects wore infrared goggles for 90 s to adjust to the dark. They were then seated on a comfortable chair and instructed to relax and focus on a light-emitting diode directly in front of them. The sampling rate for recording a pupil was 25 Hz. Customized software enabled the graphic display of pupil behavior in eight 82-s segments, and the pupillary unrest index (PUI) was calculated for each segment. To calculate the PUI, the mean of 16 consecutive data points was calculated, as well as the difference from the mean of the following 16 data points. This procedure was applied to the entire data set. All differences (in millimeters) were summed up and calculated per minute; consequently, the instability of the pupil diameter produced high values in PUI. Artifacts caused by factors such as eye movements or eye blinks were detected and removed automatically based on a physiological limit of the rate of change of pupil diameter, and an algorithm was run to substitute such interrupted recording values by linear interpolation. Details of this methodology have been validated and published in other reports [6, 7].

The relative pupillary unrest index (RPUI) was also calculated by dividing the PUI by the baseline pupil diameter, i.e., the mean pupil diameter during the first segment (82 s) of the eight consecutive segments [7].

The average pupillary diameter (PD) for the entire record (11 min, 8 segments) was calculated, and we used the average of pupillometric parameters measured twice for analysis.

Measurement of SL

Sleep latency was obtained by the two-nap sleepiness test (TNST), a modified version of the standard MSLT. The usefulness of this method was confirmed [4, 18, 19].

The subjects reclined on beds and took two naps at around 10:00 a.m. and 12:00 a.m. The test included ten electroencephalographic channels (F3, F4, C3, C4, P3, P4, O1, O2, T3, and T4), two channels for electrooculography (horizontal and vertical), and a chin electromyogram. All channels were recorded simultaneously by Neurofax EEG-1518 (NIHON-KOHDEN, Tokyo, Japan). Sleep-stage scoring was performed in 30-s epochs according to the criteria of Rechtschaffen and Kales [20]. SL was scored as the elapsed time (in minutes) from lights out to the first 30-s epoch scored as sleep. SL was calculated by averaging the times from the TNST.

Epworth Sleepiness Scale

All subjects completed the Epworth Sleepiness Scale (ESS) before the test. ESS is a self-administered questionnaire and the most widely used method to estimate the likelihood of dozing off in eight different real-life situations. It reflects individual sleep propensity [21].

Experience of a drowsy driving accident

Subjects were asked by the questionnaire whether or not they had experienced any drowsy driving accident during the past 3 years irrespective of its severity.

Data analysis

Pupillary variables (PD, PUI, and RPUI), SL, and ESS were examined for normality by Shapiro–Wilks test, and appropriate transformations were used to achieve a normal distribution as applicable. Because of the skewed distribution of PUI, RPUI, and SL, data were normalized by logarithmic transformation. All statistical analyses were performed on transformed variables when applicable. Pearson's correlation coefficient was used to examine relationships between variables. Multiple regression analysis was performed to examine the relationship of PUI and RPUI with the other variables. The difference between those who experienced a drowsy traffic accident and those who did not as measured by PUI, RPUI, and SL was determined using Student's t test. Statistical analyses were performed on a personal computer with the SPSS program package (ver. 19; SPSS, Chicago, IL), with $p < 0.05$ considered to be significant.

Table 1 Profiles and outcome measures for subjects

Outcome measure	Mean	SD
Age (years)	38.9	11.3
Height (cm)		
Male	171.6	5.3
Female	158.6	6.5
Weight (kg)		
Male	72.4	14.1
Female	52.3	4.1
Body mass index	24.1	4.4
SL (s)	203.3	129.6
PD (mm)	6.44	0.80
PUI (mm/min)	5.39	1.90
RPUI	0.83	0.30
ESS	13.8	4.0

SL Sleep latency, *PD* pupil diameter, *PUI* pupillary unrest index, *RPUI* relative pupillary unrest index, *ESS* Epworth Sleepiness Scale

Results

Profiles of subjects and descriptive statistics on outcome measures are provided in Table 1, and Pearson correlation coefficients between these measures are shown in Table 2. While the two pupillometric measures, PUI and RPUI, were highly correlated with each other ($r = 0.936$, $p < 0.01$), they showed a clear distinction in relation to PD, i.e., RPUI was inversely correlated with PD ($r = -0.431$, $p < 0.01$), while PUI did not correlate with PD at all ($r = -0.109$, $p > 0.05$). Additionally, the correlation coefficient between PUI and SL ($r = -0.402$, $p < 0.01$) was considerably higher than that between RPUI and SL ($r = -0.322$, $p < 0.05$).

Multiple regression analysis was performed using the model with PUI or RPUI as a dependent variable, and sex, age, SL, PD, and ESS were entered in each model as independent variables. As presented in Tables 3 and 4, PUI was mainly predicted by SL ($p = 0.008$), and the other variables did not significantly relate to PUI. On the other hand, RPUI was explained mainly by PD ($p = 0.001$), followed by SL ($p = 0.18$), and the other variables did not relate to RPUI. ESS did not relate to any other physiological sleepiness measures (Tables 2, 3, 4).

Objective and subjective measures of sleepiness were compared between the two groups, classified by having or not having a drowsy driving accident within 3 years before the study (Table 5). SL was significantly shorter ($p = 0.036$), and both PUI ($p = 0.004$) and RPUI ($p = 0.014$) were larger in the group of subjects that had experienced an accident than in the group that had not. There was no difference in ESS scores between the two groups.

In Fig. 1, a scatter plot is presented showing the association between PUI and SL, with different symbols for individuals with/without a self-reported experience of drowsy traffic accidents.

Table 2 Pearson's correlation coefficients

Outcome measure	SL	PUI	RPUI	PD	ESS
Age	−0.015	0.021	0.110	−0.264	−0.091
SL		−0.402**	−0.322*	−0.010	−0.062
PUI			0.936**	−0.109	−0.171
RPUI				−0.431**	−0.173
PD					0.011

All analyses were performed on transformed data

** $p < 0.01$, * $p < 0.05$

Table 3 Multiple regression analysis (forced entry method)

Independent variables	Partial regression coefficient	Standardized partial regression coefficient	p values	95 % CI	
Constant	1.459		0.000	0.915	1.632
Sex	−0.060	−0.146	0.303	−0.177	0.056
Age	−0.054	−0.192	0.179	−0.134	0.026
SL	−0.198	−0.392	0.008	−0.340	−0.055
PD	−0.028	−0.157	0.268	−0.078	0.022
ESS	−0.007	−0.198	0.160	−0.017	0.003
Dependent variable: PUI					

$p = 0.027$, by analysis of variance (ANOVA)

CI Confidence interval

Multiple correlation coefficient adjusted for the degree of freedom $R^2 = 0.175$

Table 4 Multiple regression analysis (forced entry method)

Independent variables	Partial regression coefficient	Standardized partial regression coefficient	p values	95 % CI	
Constant	0.959		0.000	−0.480	1.437
Sex	−0.050	−0.116	0.378	−0.164	0.064
Age	−0.047	−0.159	0.230	−0.125	0.031
SL	−0.169	−0.320	0.018	−0.309	−0.030
PD	−0.087	−0.470	0.001	−0.136	−0.038
ESS	−0.007	−0.191	0.146	−0.017	0.003
Dependent variable: RPUI					

$p = 0.002$, by ANOVA

Multiple correlation coefficient adjusted for the degree of freedom $R^2 = 0.284$

Discussion

Of the pupillometric measures of sleepiness, PUI was significantly correlated with SL ($r = -0.402$; Table 2) and was also mainly explained by SL (Tables 3, 4) in the multiple regression analysis. These results are in agreement with those of other reports [12, 13].

The locus coeruleus (LC), one of the central noradrenergic neurons located in the brainstem, projects to both the cerebral cortex and to the Edinger–Westphal nucleus (EWN) of the midbrain. The projection to the cortex has been implicated in the maintenance of alertness, whereas the projection to the EWN has been related to an inhibitory influence on the parasympathetic light reflex. In addition, there are different descending sympathetic fibers from the LC that contribute to the control of sympathetically mediated pupil dilation [22]. Consequently, it is reasonable to suppose that the LC constitutes a link between the level of arousal and pupil size and that the fluctuations in LC activity may underlie PU.

The close relationship between PUI and SL observed in this study, therefore, might reflect the underling neurophysiological connection between pupillary oscillation mechanisms and central nervous activation.

Although RPUI, another PU outcome we found to be highly correlated with PUI ($r = 0.936$; Table 2), behaved in a manner similar to PUI in terms of the relationship with SL, the Pearson's correlation coefficient with SL in Table 2 ($r = -0.322$, $p = 0.031$) was slightly lower than that of PUI. The correlation coefficient presented improved following adjustment of the PD ($r = -0.362$, $p = 0.016$). In addition, RPUI was predominantly predicted by PD, followed by SL among the independent variables in the multiple regression analysis (Tables 3, 4). Accordingly, it would be reasonable to conclude that when assessing physiological sleepiness by pupillometer, PUI is preferable to RPUI as a valid pupillary index. However when RPUI, which is a default output of the portable electronic pupillometer F2D, is used, it is advisable to adjust RPUI by PD in the inter-individual comparisons.

Table 5 Comparison of measures of sleepiness between study subjects who had experienced a driving accident while drowsy during the past 3 years and those who had not

Outcome measures	Experience of drowsy driving accidents ($n = 4$)	No experience of drowsy driving accidents ($n = 41$)	p values
Age	32.5 (15.2)	39.6 (10.9)	0.236
SL	1.95 (0.34)	2.25 (0.26)	0.036
PD	6.63 (1.03)	6.42 (0.79)	0.604
PUI	0.895 (0.120)	0.690 (0.131)	0.004
RPUI	0.063 (0.160)	−0.126 (0.139)	0.014
ESS	14.8 (3.1)	13.7 (4.1)	0.606

All analyses were performed on transformed data

Data are presented as the mean with the standard deviation in parenthesis

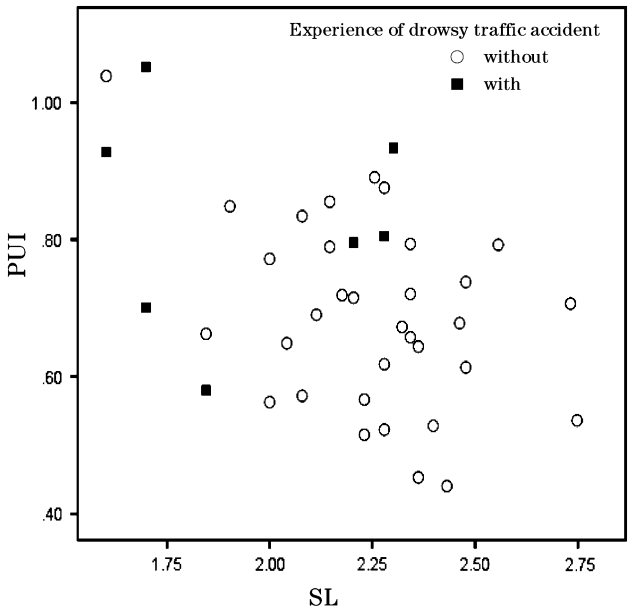

Fig. 1 A scatter plot showing the association between the pupillary unrest index (*PUI*) and sleep latency (*SL*)

PD correlated neither with SL nor ESS, indicating that this pupillometric parameter is not suitable for detecting inter-individual differences in sleepiness, while in intra-individual circadian variations [16], it correlated well with MSLT-derived sleepiness, which seems to reflect the same aspect of the level of tonic central nervous activation. Morrell [23] indicated that both SL and ESS reduced with age in patients with moderate-to-severe sleep disordered breathing, and Eggert [24] found PUI and PD to decrease with increasing age in healthy subjects. In our study, however, age did not correlate with any objective or subjective sleepiness measures (Table 2). However, all of our subjects were under 60 years of age; therefore, the association between pupillometric parameters and SL should be further clarified.

One of the most serious outcomes associated with sleepiness is motor vehicle accidents [25]. Increased accident rates among patients with sleep disorders, such as obstructive sleep apnea [26], insomnia [27], and narcolepsy [28], have been demonstrated. Drake [29] also demonstrated that individuals with MSLT values of ≤5 were at an increased risk of being involved in a car crash over the course of a 10-year assessment period in a general population sample.

Although the possibility of self-report bias and inaccurate recall might exist, four subjects (sleep apnea, narcolepsy, hypersomnia, and insufficient sleep syndrome, respectively) reported having had a drowsy driving accident during the previous 3 years. In addition, a significant difference in PUI, RPUI, and SL were observed between the accident/no accident groups. We could not obtain any dose-response-like relationship between accidents and physiological measurements because of the small number of subjects in this study. When more easily applicable pupillometric methods and measures are developed and their reliable normal values are set, it will be possible to validate the sensitivity and specificity of the tests for driving accidents by studies on a larger number of individuals. Such validated measures will greatly contribute toward screening individuals at an increased risk for drowsy driving accidents and for interventions intended to minimize this risk among, for example, commercial drivers or those in the transportation industry.

ESS is the most widely used standardized subjective measure of sleepiness [21]. In our study, however, the score of this scale did not correlate with any of the other objective sleepiness measures, i.e., SL, PUI, and RPUI (Tables 2, 3, 4). There was also no significant difference in ESS scores between the drowsy traffic accident/no drowsy traffic accident groups (Table 5). These findings are consistent with those of other studies reporting either no correlation or a low correlation between the subjective ratings of sleep propensity on the ESS and the average SL in clinical and nonclinical samples [2, 30]. The lack of a significant association between ESS and objective sleepiness measures may be due to several factors. First, as the ESS score surpassed 10 in almost all of our subjects, i.e., 40 out of 45 patients in our study, the ceiling effect of ESS might have reduced its differential sensitivity, resulting in poor correlations with SL or pupillometric parameters. Second, ESS and the other objective physiologic sleepiness measures reportedly characterize different aspects of sleepiness [31, 32]; that is, ESS assesses more global sleepiness, while the other measures (MSLT and pupillometry)

might reflect both trait- and state-like sleepiness. Finally, it is possible that many individuals are not able to accurately assess their own level of alertness [29]. Our findings highlight the advantage of having a physiological assessment of sleepiness with a narrow range of differences among the subjects.

Limitations

All of the subjects were patients at the Sleep Disorders Center and were representative of a heterogeneous group of sleep disorders, such as sleep apnea and narcolepsy, where the underlying neurophysiological mechanisms might be distinct [13, 33]. In addition, a considerable difference in age distribution was observed among conditions; for example, the patients with sleep apnea were older than those with narcolepsy. Because of the small number of subjects enrolled in the study, we could not properly control these confounding factors and could not fully avoid possible biases of the results. Therefore, care must be taken in applying our conclusions to patients with any specific sleep disorder or to a healthy general population.

In some of our subjects, medication could not be discontinued before starting the study due to clinical reasons. The effects of some drugs, therefore, might somehow confound the relationship between the outcome measures of the study. As for the pupillary effects of the drugs, changes both in pupillary measurements and central alertness levels have been simultaneously investigated for antidepressants [34], central α2-adrenoceptor agonists and antagonists [22], anti-Parkinsonian drugs [35], benzodiazepan [36], modafinil [37], and anti-hypertensive drugs [38]. In these studies, the direction and amplitude of changes in pupillary measurements or central alertness varied depending on the effect of each drug. However, a combination of increased (reduced) changes in pupillary oscillations and reduced (increased) changes in central alertness was indicated in most of the studies.

The measurement of pupillometric parameters and SL was conducted only twice per subject before noon, and an average value was calculated from the two sessions for analysis. Therefore, the association between pupillometry and MSLT could not be adjusted for the time-of-day effects [13, 16] in this study.

Despite these limitations, our results indicate that in our study cohort the pupillometric sleepiness measure, PUI, was significantly correlated with, and behaved in a manner equivalent to MSLT-derived SL. Several points remain to be carefully examined, such as establishing a normative standard of PUI before applying pupillometry for screening sleepiness in a general population or in occupational settings.

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Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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