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Valid parameters for investigation of the pupillary light reflex in normal and diabetic subjects shown by factor analysis and partial correlation

R. H. Straub[1], U. Thies[2], A. Jeron[2], K.-D. Palitzsch[1], J. Schölmerich[1]

[1] Department of Internal Medicine I, University Hospital, Regensburg, Germany
[2] Department of Internal Medicine, University Hospital, Freiburg, Germany

Summary Pupillary test data of 103 normal and 119 diabetic subjects (47 IDDM, 72 NIDDM) were evaluated by factor analysis. From a total of nine pupillary parameters three factors were extracted in the analysis. Factor 1 represents maximal pupillary area, contraction velocity at 1 s, dilation velocity at 6 s and minimal pupillary area – static and simple dynamic parameters; factor 2 amplitude of pupillary unrest, area under the detrended curve of pupillary unrest and period of pupillary unrest – parameters of pupillary unrest; factor 3 fusion frequency of pupillary response following flicker stimuli and latency time of pupillary light reflex – second order dynamic parameters. Factor analysis was then applied to investigate diabetic patients with a high percentage of autonomic neuropathic participants (about 39% had pupillary and about 35% had cardiorespiratory function disorders), which revealed the same three factors as those identified in normal subjects. Furthermore, an age-related database of parameters of pupillary unrest is given. It demonstrates that normal subjects and diabetic patients did not differ in the period of pupillary unrest (normal vs diabetic (mean ± SEM): 1550 ± 29 vs 1536 ± 27 ms; $2p > 0.5$). The difference in amplitude (47.8 ± 2.8 vs 41.0 ± 2.6% percentile; $2p = 0.071$) and area under the detrended curve of pupillary unrest (47.9 ± 2.8 vs 40.8 ± 2.6% percentile, $2p = 0.062$) seems to show a trend but was not significant. In conclusion, factor analysis revealed three different pupillary test factors. From the comparison of normal and diabetic subjects factor 1 which accounts for the highest percentage of variance (≅ 43%) and factor 3 (≅ 12%) appear to be useful for investigating the pupillary light reflex. Factor 2 is not useful because of the insignificant differences between the normal and diabetic group. From factor analysis and partial correlation we believe that pupillary autonomic function in diabetic patients can be best assessed by using only two parameters, maximal pupillary area and latency time. [Diabetologia (1994) 37: 414–419]

Key words Pupillary autonomic function, pupillary parameters, factor analysis, pupillary unrest.

Examination of the pupillary light reflex is a component of the test battery used to assess autonomic nervous system abnormalities in diabetic patients [1–5]. Because many different pupillary parameters must be tested, a complete investigation of the pupillary control system is very time-consuming. The aim of the present study was to reduce the pupillary test battery and to obtain a minimum of meaningful parameters. Factor analysis and partial correlation were used to achieve this purpose.

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Corresponding author: Dr. R. H. Straub, Department of Internal Medicine I, University Hospital, D-93042 Regensburg, Germany

Abbreviations: IDDM, Insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; lx, lux; lm, lumen

Subjects and methods

Subjects

The study group comprised 222 subjects (103 normal, 47 IDDM and 72 NIDDM study subjects). Table 1 gives the basic clinical parameters of normal subjects and diabetic patients. Normal

Table 1. Clinical parameters of normal subjects and diabetic patients

	n	Age (years)	Men/women	IDDM/NIDDM	Duration of disease (years)
Normal subjects	103	39.0 ± 1.6[a] [14–75]	55/48		
Diabetic patients	119	51.1 ± 1.5 [14–80]	52/67	47/72	16.7 ± 1.0 [0.5–62.0]

[a] Normal subjects are younger than diabetic patients, $2p < 10^{-5}$
Values are given in mean ± SEM. Ranges are given in brackets

Table 2. Abbrevations, units and meanings of the investigated pupillary parameters

Abbreviation	Unit	Meaning
Static and simple dynamic parameters		
MaPA	mm^2	Maximal pupillary area in darkness
CV1	mm^2/s	Contraction velocity at 1 s
DV6	mm^2/s	Dilation velocity at 6 s
MiPA	mm^2	Minimal pupillary area
Parameters of pupillary unrest		
Ampl	mm^2	Amplitude of pupillary unrest after detrending the data set
AUC	$mm^2 \cdot s$	Area under the detrended non-smoothed curve of pupillary unrest
P	ms	Period of pupillary unrest derived from the first nadir of autocorrelation
Second order dynamic parameters		
LT	ms	Latency time of pupillary light reflex
FF	Hz	Fusion frequency of pupillary response following flicker stimuli

subjects were included as a part of an earlier study [6]. Diabetic patients were out-patients of the Department of Internal Medicine of the University Hospitals of Freiburg ($n = 77$) and Regensburg ($n = 42$). All subjects were fully informed of the purpose of the study and gave their written consent to participate. Five standard cardiorespiratory function tests according to the method of Ziegler et al. [7] showed that about 35% of the diabetic patients had abnormalities in two or more tests (data not shown). The standard pupillary tests of latency time, maximal pupillary area, contraction velocity at 1 s and dilation velocity at 6 s were abnormal (= below the 5% percentile) in more than 39% of the patients. Visual acuity was greater than 0.5 in all subjects. On the day before and on the day of testing subjects were asked to abstain from sleep reduction, extreme physical and emotional stress, coffee, cigarettes or other factors influencing autonomic function. None of the participants were taking any drug known to influence the pupil. The age of the normal and diabetic subjects is different (Table 1). Hence, separate mention of these two groups should only be used for description, and if age is corrected, for comparison.

Pupillometry

Pupillometry was performed with an infrared video camera and biometry using a video-genlock interface and computer-based image-analysing system [8]. The pupillometric procedure has been previously described [6]: for dark adaptation, subjects rested in an upright position for 10 min in darkness (light intensity less than 1 lx). To prevent accommodation they looked at a point at a distance of 5 m. The video recorder was then started to record the images of the pupil (0^{th} s). After 10 s, a light stimulus with an intensity of 175 lx was given to provide the closed-loop technique of retinal illumination (exact description of the light stimulus in 8). After another 10 s, the illumination ended (20^{th} s) and the dilation of the pupil was recorded for further 20 s. During constant illumination after reaching the minimal pupillary area and after a small period of minimal redilation between the 14^{th} and 20^{th} s, pupillary unrest was investigated (observation period = 6 s).

Fusion frequency of pupillary response following flicker stimuli was investigated by direct observation of the pupil while stimulating with an increasing flicker frequency. The light stimulus intensity was also 175 lx. The frequency at which the pupil does not respond to flickering is called fusion frequency. The method has been previously described in detail [3]. Fusion frequency was not measured in diabetic subjects because this is a time-consuming procedure. Table 2 shows the investigated pupillary parameters, units and abbreviations.

Pupillary unrest was investigated using the following procedures: during an observation period of 6 s (14^{th}–20^{th} s) pupillary area was measured every 120 ms (50 values = 8.33 Hz sampling rate). The smoothing algorithm which involves a repeated sequence of moving averages of three ($Y(I) = (Y(I-1) + Y(I) + Y(I+1))/3$) and hanning ($Y(I) = (Y(I-1) + 2 \cdot Y(I) + Y(I+1))/4$) was used [9]. For stationarization, the data were detrended by subtraction of a polynomial trend of the sixth order. Autocorrelation was applied to identify a significant regular oscillation of the pupillary area during pupillary unrest [10]: the autocorrelation coefficients were calculated between the original data set against time and sequential 'copies' of the data generated by moving the original data by increments of one time step (= lag time = 120 ms). The initial correlation is defined as $r = 1.000$ and the autocorrelation coefficient then decreases to reach the first nadir when the data is 180° out of phase ($r < 0$). The autocorrelation coefficients were plotted against lag time to produce correlograms. The period of oscillation is defined as the lag time to the first significant minimum in the correlogram multiplied with two ($2p$ for autocorrelation coefficient < 0.05). The first minimum was significant in every examined subject

Table 3. Factor analysis of pupillary parameters derived from 103 normal subjects and 119 diabetic patients (in brackets)

Final statistics of the principal-components analysis			
	Eigenvalue	% of variation	Cumulative percentage
Factor 1	3.841 [3.270]	42.7 [40.9]	42.7 [40.9]
Factor 2	1.834 [2.154]	20.4 [26.9]	63.0 [67.8]
Factor 3	1.077 [1.085]	12.0 [13.6]	75.0 [81.4]
Factor matrix after varimax rotation			
	Factor 1	Factor 2	Factor 3
MaPa	0.943 [0.969]		
CV1	0.855 [0.951]		
DV6	0.814 [0.948]		
MiPA	0.796 [0.618]		
Ampl		0.925 [0.947]	
AUC		0.925 [0.959]	
P		0.675 [0.551]	
FF			−0.817 [--]
LT			0.580 [0.881]

For abbreviations see Table 2. The different pupillary parameters load variously on three factors. FF was not included in the analysis of diabetic patients because it was not measured. Factor loadings less than 0.500 are not shown

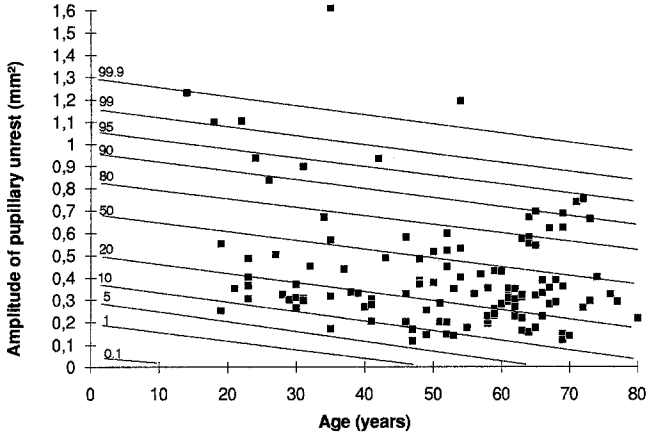

Fig. 1. Amplitude of pupillary unrest of all diabetic subjects studied. The percentiles of normal subjects are given (*solid lines*). Only 7 of 119 participants (5.9%) were below the 10% percentile but no subject was below the 5% percentile. The equation of the regression line concerning diabetic subjects is: Amplitude = 0.64–0.0042·age; with $r = -0.245$, $2P = 0.007$, $n = 119$. The amplitude of pupillary unrest in diabetic subjects is seen to be age-dependent

($n = 222$). The first maximum was only significant in 158 subjects (71.2%). Period derived from the first minimum and period derived from the first maximum were strongly correlated ($r = 0.998$, $2p < 10^{-5}$). These facts allow the first minimum to be the important parameter for the calculation of the period of pupillary unrest. The mean amplitude of oscillation for each data set was calculated as the root mean square of the detrended non-smoothed data set multiplied with $2 \cdot \sqrt{2}$ [10]. As an another measure, we used the area under the detrended non-smoothed curve which was calculated as the sum of the single values of the detrended data set of every subject multiplied with the total observation time.

Statistical analysis

For *factor analysis* and *partial correlation* SPSS/PC$^+$ Advanced Statistics were used [11]: the sampling adequacy for each variable was examined on the anti-image correlation matrix and all variables had high scores (0.579–0.877). The validity of the factor analysis for the data was assessed by the Kaiser-Meyer-Olkin measure of sampling adequacy which was 0.68. The hypothesis that the correlation matrix is an identity matrix was rejected by the Bartlett's test. Factors were then extracted by the principal-components technique. Factors with an eigenvalue greater than 1 were included and a varimax-rotation was used to enhance factor loading.

For *autocorrelation* and *smoothing* Forecasting and Time Series of Lionheart Press, Inc., was used ($2p$ for autocorrelation coefficient < 0.05, [9]).

The relationship between age and parameters of pupillary unrest is expressed mathematically by linear-regression analysis. Comparisons between normal and diabetic subjects were made using the Mann-Whitney U-test for independent samples.

Results

Table 3 shows the result of factor analysis in normal and diabetic subjects. In data from normal and diabetic subjects three factors were extracted in the analysis. They account for 75% of total variance in normal and 81% in diabetic subjects. Factor 1 represents simple static and dynamic pupillary parameters such as maximal pupillary area, contraction velocity at 1 s, dilation velocity at 6 s and minimal pupillary area, which account for the highest percentage of variance (42.7% in normal and 40.9% in diabetic subjects), factor 2 parameters of pupillary unrest such as amplitude, area under the detrended curve and period of pupillary unrest, and factor 3 second order dynamic pupillary parameters such as fusion frequency and latency time. In diabetic patients, the analysis revealed the same factors with nearly the same percentages of variance.

Table 4 summarizes the correlation between age and parameters of pupillary unrest. The regression parameters a and b, the correlation coefficient r and its p-value of the period and amplitude of pupillary unrest are given. Furthermore, a formula to calculate the exact percentile localization of every individual tested person is given in Table 4. It is evident that the period of pupillary unrest is age-independent ($2p = 0.661$) and

Table 4. Correlation between age and parameters of pupillary unrest from 103 normal subjects

Parameter	Unit	Mean ± SD	a	b	r	p
Ampl	[mm²]	0.509 ± 0.24	0.676	−0.00427	−0.283	0.004
P	[ms]	1547.2 ± 290.5	1516.2	0.793	0.044	0.661
Parameter		syx	M	Qx		n
Ampl		0.233	39.03	26230.9		103
P		291.67	39.03	26230.9		103

$$t = \left| \frac{\text{value} - (a + b*\text{age})}{\text{syx}* \left(1 + \frac{1}{n} + \frac{(\text{age} - M)}{Qx}\right)^{0.5}} \right|$$

a and b, Linear-regression parameters; *r*, correlation coefficient; value, individual value of a patient; age, individual age of a patient; syx, M, Qx and n are statistical parameters [6]. Using the given formula, it is possible to calculate the exact percentile localization. The *p*-value of the t value with *n*-1 degrees of freedom gives the exact percentile. For other abbreviations see Table 2

Table 5. Matrix of partial correlation coefficients derived from 103 normal and 119 diabetic (in brackets) subjects

	P	AUC	Ampl	LT	FF	MaPA	CV1	MiPA
AUC	−0.041 [0.392]ᵇ							
Ampl	0.092 [−0.351]ᵇ	0.992ᵇ [0.990]ᵇ						
LT	−0.137 [0.036]	−0.139 [−0.062]	0.144 [0.072]					
FF	−0.094	−0.261ᵃ	0.271ᵃ	−0.063				
MaPA	−0.044 [0.066]	−0.079 [−0.091]	0.067 [0.068]	−0.110 [−0.013]	0.074			
CV1	−0.039 [−0.130]	0.086 [0.238]ᵃ	−0.060 [−0.209]ᵃ	−0.112 [0.227]ᵃ	0.078	0.713ᵇ [0.634]ᵇ		
MiPA	0.031 [−0.095]	0.121 [0.158]	−0.106 [−0.086]	0.205ᵃ [−0.229]ᵃ	−0.146	0.701ᵇ [0.394]ᵇ	−0.332ᵇ [−0.285]ᵇ	
DV6	0.068 [0.112]	0.036 [−0.201]ᵃ	−0.035 [0.186]	0.121 [−0.120]	0.083	0.425ᵇ [0.373]ᵇ	0.134 [0.390]ᵇ	−0.269ᵇ [0.044]

ᵃ $2p < 0.05$; ᵇ $2p < 0.01$. For abbreviations see Table 2. FF was not measured in diabetic subjects

amplitude of pupillary unrest age-dependent ($2p = 0.004$). In diabetic patients, amplitude of pupillary unrest was also age-dependent (Fig. 1). Furthermore, no diabetic patient had a test result which was below the 5% percentile of normal subjects. The difference in the mean percentile localization of amplitude (47.8 ± 2.8 vs 41.0 ± 2.6% percentile; $2p = 0.071$) and area under the detrended curve of pupillary unrest (47.9 ± 2.8 vs 40.8 ± 2.6% percentile, $2p = 0.062$) was not significant. The period of pupillary unrest did not differ between diabetic and normal subjects (normal vs diabetic (mean ± SEM): 1550 ± 29 vs 1536 ± 27 ms; $2p > 0.5$). Strictly speaking, no diabetic subject is affected by autonomic disorders concerning pupillary unrest. However, pupillary autonomic function disorders, defined as a test result below the 5% percentile of normal subjects, obviously occur in the long-term diabetic study subjects when considering maximal pupillary area (diabetic subjects with a test result below the 5% percentile: 40.3%; between 5% and 10% percentile: 10.9%), contraction velocity at 1 s (47.9%; 9.2%), dilation velocity at 6 s (46.2%; 13.4%), minimal pupillary area (27.7%; 55.5%), and latency time (36.1%; 12.6%).

The matrix of partial correlation coefficients from normal and diabetic subjects revealed a high interrelation between amplitude of pupillary unrest and area under the detrended curve of pupillary unrest which stems from the mathematical relationship between the two parameters; the amplitude is directly proportional to the area under the detrended curve of pupillary unrest (Table 5). Furthermore, the amplitude and the period of pupillary unrest are forming the pupillary oscillation independently from each other in normal subjects but not in diabetic patients. This means in diabetic subjects, period and amplitude are significantly inversely correlated, i. e., if the amplitude is small the period is prolonged.

Maximal pupillary area correlates highly significantly with contraction velocity at 1 s, minimal pupillary area and dilation velocity at 6 s in normal and diabetic subjects, and latency time correlates with minimal pupillary area in normal subjects and with contraction velocity at 1 s and minimal pupillary area in diabetic patients (Table 5).

Discussion

Using nine different parameters to assess pupillary control system both normal and diabetic subjects have been investigated. Factor analysis was applied to explore common test groups of pupillary function. Three factors were extracted in normal and diabetic subjects representing three different test groups. The factors indicate that certain test groups are more important and they can be listed in a descending order: factor 1 represents a test group of static and simple dynamic parameters, factor 2 represents pupillary unrest parameters and factor 3 second order dynamic parameters. Factor analysis proved to be a good statistical technique to differentiate between factors as has been shown for disease patterns in patients with systemic lupus erythematosus [12]. After receiving the statistical factors, it must be shown whether these factors are meaningful for assessing pupillary dysfunction in the diabetic group and whether the parameters which are forming the factor are independent from each other. It was shown that parameters forming factor 1 and factor 3 differentiate clearly between normal and affected subjects using the 5% percentile localization. But, parameters forming factor 2 were completely normal in the diabetic group. These results are in contrast to a previous investigation of pupillary unrest in diabetic patients [4]. These authors found a reduced pupillary unrest in diabetic patients and explained this finding as an autonomic nervous system abnormality. They used the standard deviation of the original measurements taken with a 10-Hz sampling rate in darkness and in brightness [13]. The somewhat contrasting results may be due to 1) a different light stimulus (1 mm beam in an open-loop fashion with an intensity of 512 µlm/3.14 mm^2 ≅ 163 lx in the mentioned study [4] vs a closed-loop technique with 175 lx in our study), 2) a longer lasting illumination (15 s vs 4 s) and observation period (15 s vs 6 s), 3) different study groups (80 IDDM and 26 normal subjects vs 47 IDDM, 72 NIDDM and 103 normal subjects) and, more important, 4) to a different statistical technique (without vs with subtraction of a polynomial trend). In a preliminary investigation, we found a correlation between maximal pupillary area and non-detrended amplitude of pupillary unrest ($r = 0.351$, $2p = 0.000078$) as well as between minimal pupillary area and non-detrended amplitude of pupillary unrest ($r = 0.400$, $2p < 10^{-6}$). Hence, amplitude of the non-detrended data set seems to be statistically dependent only on maximal pupillary area. From a diagnostic viewpoint, the amplitude of the non-detrended data set does not seem to give any further information compared to the maximal pupillary area. This was also shown in a previous study [4] in which a high correlation between the parameter of unrest and pupillary area in darkness (= maximal pupillary area) was observed. Hence, a polynomial trend must be subtracted to standardize the measured areas and this should account for the differing results in the two studies. However, our results show that a difference between the two groups, at least in the form of a trend, is present. Nevertheless, it is thought that those parameters of pupillary unrest utilized here are not useful because they are not markedly changed in autonomic neuropathy. This is valid, especially, for the period of pupillary unrest which had not previously been investigated. The latter seems to stem from an absolute stable neuronal oscillator which does not change in autonomic neuropathy or during aging.

The interrelationship between the pupillary parameters in the different test groups (factors) is obvious, especially when considering factor 1. To reduce to one meaningful test, the strongly correlating parameters of factor 1 must be eliminated to obtain the one test which is easiest to perform. Maximal pupillary area seems to be the best parameter for this reason in test group 1 (factor 1). Furthermore, factor 3 can be reduced to the latency time because it is easy to measure. If a previous study [3] is also considered, fusion frequency may also be a valid parameter. Since measuring fusion frequency is somewhat more complicated and time-consuming than measuring latency time the latter should be given priority. Thus nine different pupillary parameters can be reduced to only two, maximal pupillary area and latency time. These represent different parts of the autonomic nervous system, maximal pupillary area, the sympathetic and latency time, the parasympathetic portion.

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