

Pupillographic Measurements with Pattern Stimulation: The Pupil's Response in Normal Subjects and First Measurements in Glaucoma Patients

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PURPOSE. This study was undertaken to characterize the influence of contrast, luminance, and spatial frequency of a pattern stimulus on the pupil reaction of healthy subjects. First measurements with this technique in patients with glaucoma were compared with those in a control group.

METHOD. Grating patterns were presented using a Maxwellian-view system to study series of contrast, luminance, and spatial frequency in three healthy subjects. The best two stimulus conditions were determined and were then used to examine 19 patients with open-angle glaucoma and 16 control subjects.

RESULTS. In healthy subjects, an increasing contrast led to an increase in amplitude and a decrease in latency of the pupil reflex. Increasing luminance also resulted in an increase in the amplitude. The offset component of the pupil reflex was most pronounced at low spatial frequencies and the onset component at high spatial frequencies. When healthy subjects were compared with patients with glaucoma, control subjects generally had higher amplitudes, velocity, and acceleration of pupil constriction than did the patients with glaucoma. These differences were significant when the test was performed with a spatial frequency of 6.25 cyc/deg.

CONCLUSIONS. Best stimulus conditions to elicit a pupil response to a pattern grating stimulus are 100% contrast and 55 cd/m² mean luminance. The choice of the spatial frequency determines which component of the pupil reflex is more pronounced. Differences between patients with glaucoma and healthy control subjects are demonstrable. (*Invest Ophthalmol Vis Sci.* 2006;47:4947–4955) DOI:10.1167/iovs.06-0021

Traditionally, the pupil light reflex in humans is tested by presenting light stimuli that increase the light flux entering the eye. However, in recent years it has been shown that pupil movements can be evoked with patterned stimuli that do not change the overall light level within the eye.^{1–4} Several groups presented pattern stimuli in the onset–offset mode and were

able to predict perceptual visual acuity using checkerboard or grating patterns of increasing fineness.^{5–8} Also, by alternating the spectral composition of unpatterned isoluminant stimuli, pupil reactions can be obtained.⁹ Furthermore, the presence of transient and sustained components in the pupillogram was demonstrated, evoked by achromatic spatial patterns (onset–offset) and by luminance and color stimuli.^{9,10} The investigators suggested an analogy to the function of the magno- and parvocellular layers of the lateral geniculate nucleus. Pupil responses also can be evoked by the onset of and by a change in the direction of coherent motion.¹¹

In lesions of the higher visual pathways above the lateral geniculate body, deficits in pupil reaction can occur on light stimulation of the corresponding field defects^{12,13} and in subjects with blindsight.⁴

These findings suggest that pupillary activity reflects more than just an adaptive mechanism to changes in ambient light intensity or the activity of the autonomous nervous system. It seems that the input to the pupillomotor centers is functionally similar to the visual input to the perceptual centers of the brain. Apparently, pupil responses reflect the activity of different classes of neurons of the visual pathway and are another means to study several different visual functions without having to rely on the patient's co-operation. It was the purpose of the present investigation to analyze pupil responses by onset–offset stimuli, using grating patterns of different spatial frequencies, contrasts, and intensities. In a further step, we show pupillograms in a clinical setting: As patterned stimuli have been proven useful in glaucoma diagnostics, we tested a group of patients with glaucoma and compared their pupillograms with those of a healthy control group.^{14,15}

METHODS

The Stimulus

The stimulator was a two-channel Maxwellian view system with a Xenon high-pressure arc lamp as the source of white light (Fig. 1A).

To study pupil reaction to pattern offset–onset stimuli in three healthy subjects, channel 1 provided vertical square-wave stripe patterns with the fundamental spatial frequencies of 0.33, 0.88, 1.2, 2.7, 3.45, 6.25, and 9.2 cyc/deg. When channel 2 was closed, the patterns were seen with a contrast of 100%. By opening channel 2, the contrast was reduced by superimposing homogeneous light on the pattern. The luminance and the contrast of the stimulus were adjusted by the appropriate choice of neutral-density filters in both channels.

To study the pupil reaction in patients with glaucoma and a healthy control group, we used spatial frequencies of 0.33 and 6.25 cyc/deg, a mean luminance intensity of 55 cd/m², and a contrast of 100%. As will be explained later, these stimulus conditions evoked the best pupil responses in the three healthy subjects.

The diameter of the circular stimulus field was 32° and cross hairs provided a central fixation mark. Thus, the point of fixation was always at the edge of a bright stripe, as shown in Figure 1B. Both the patterns and the fixation mark were viewed at infinity (i.e., with relaxed

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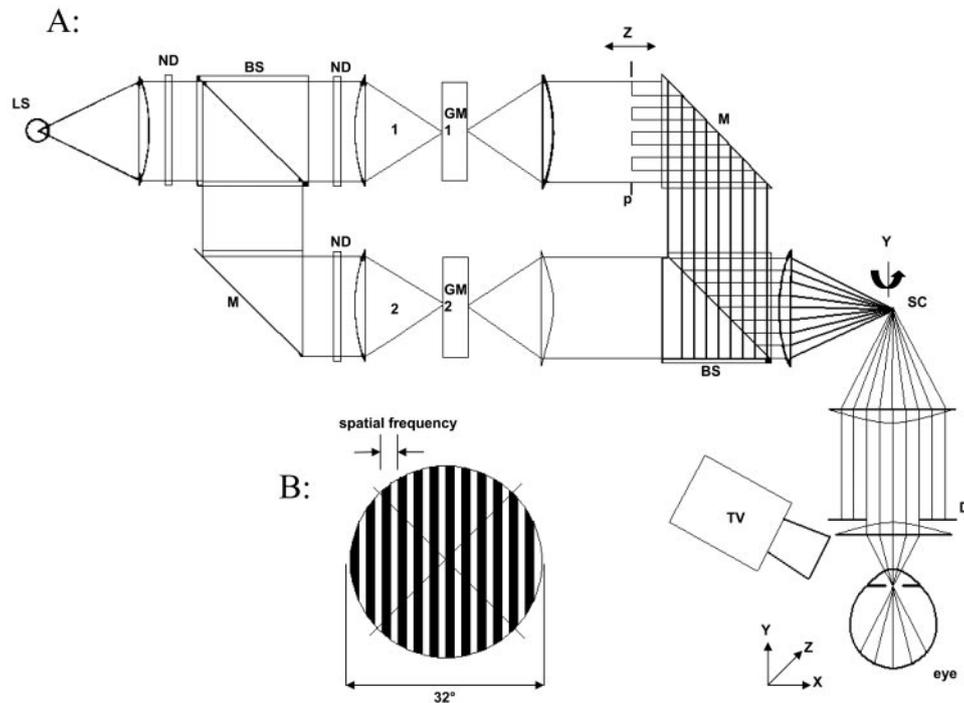


FIGURE 1. (A) Schematic drawing (not scaled) of the Maxwellian-view system used to present the stimulus. 1, channel 1; 2, channel 2; LS, light source; ND, neutral-density filters; BS, beam splitters; M, mirror; GM1 and GM2, grating monochromators (white light was used in all recordings and both channels); P, slide with stripe pattern; SC, scanner; D, diaphragm for adjustment of the field of stimulation to 32° ; eye (the chin rest can be moved along the x -, y -, and z -axes). TV, infrared video CCD camera. With proper use of neutral-density filters, the contrast and the intensity of the stimulus could be adjusted. Channel 2 was blocked in 71 recordings of patients with glaucoma or ocular hypertension and control subjects, to achieve 100% contrast. (B) Stimulation field of 32° as seen by the subjects during the onset: black-white pattern and cross hairs providing a fixation mark.

accommodation, they were sharply focused within the eye). Thus, there was very little chance that accommodation would reduce the pupil width. The Maxwellian view provided "open-loop" stimulation, meaning that movements of the pupil occurring with pattern stimulation had no effect on the retinal illumination. Equal luminance during onset and offset had been verified with a photometer.

To provide onset-offset stimulation we used a vibrating scanner,¹⁶ as demonstrated in Figure 1. In short, before entering the eye, the combined beams of the viewing system were focused on and reflected from a front-surface mirror mounted on the vertical axis of a servocontrolled galvanometer (CCX 101; General Scanning Inc., Watertown, MA). Rotations of the scanner axis led to horizontal movements of the pattern. Vibrating the scanner at a frequency above subjective flicker fusion made the pattern virtually invisible (offset); stopping the vibration made the pattern visible (onset). Scanner movement was controlled by a personal computer through an analog-digital (AD) converter. In the experiments, offset and onset were both 2000 ms. The overall luminance during offset and onset periods did not change. The position of the pattern in the stimulus field was adjusted so that a border of a stripe was always superimposed with the fixation cross. With each onset of the pattern, its spatial phase was changed by 180° .

Recording

Pupillography was performed by using a customer-designed infrared video CCD camera (HEY Headbased Eyetracker; AMtech, Weinheim, Germany) mounted in front of the stimulated eye next to the final lens of the Maxwellian-viewing system (Fig. 1). The processor of the recording system detected the center of the dark pupil and was programmed to follow small movements of the eye. The infrared image of the eye and the output of the pupillograph were constantly monitored on a TV set and on an oscilloscope, respectively. The output voltage of the pupillographic system was calibrated in square millimeters of pupil area by placing, at the focal point of the viewing system, artificial pupils printed as black circles of known area on white cardboard.

The pupil signal was fed via an AD converter into the same computer that controlled for scanner movement. A homemade Pascal-written program was used for data analysis. The averaged ($n = 30$) records were stored on disk and subsequently evaluated. In all experiments, the sampling rate of the computer was 250 Hz, the sweep

length 4000 ms. Parameters of the pupil response studied are shown in Figure 2.

The surrounding illumination in the experimental room was dimmed to approximately 2 cd/m^2 . Measurements started after at least 3 minutes of adaptation to the dim room illumination.

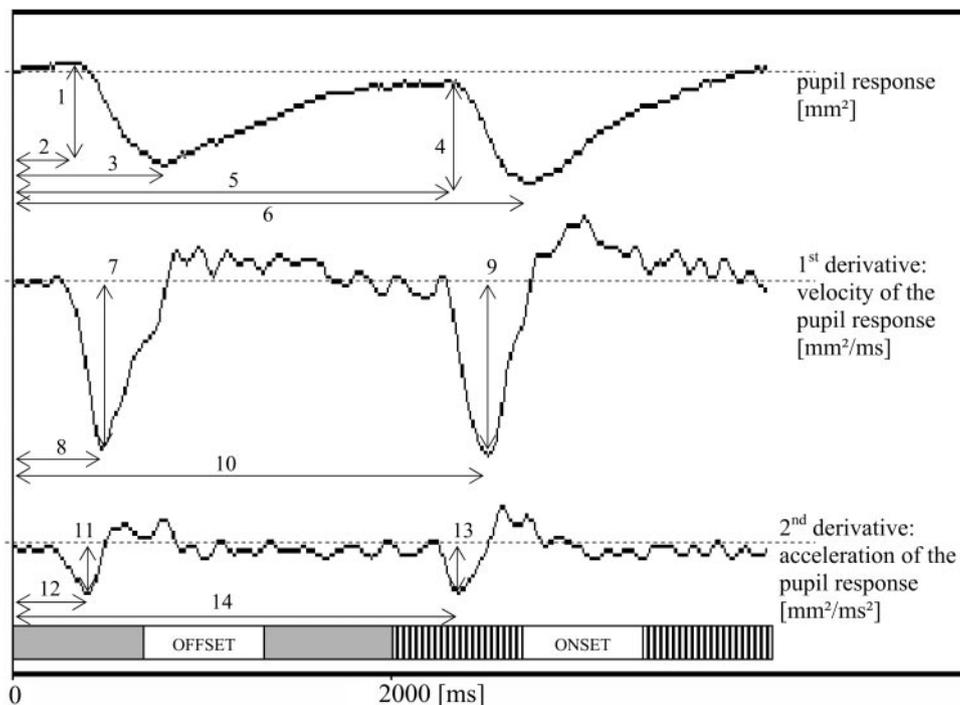
Subjects and Procedures

To study the influence of contrast, we performed luminance and spatial frequency complete series in the right eyes of three healthy subjects aged 47, 60, and 61 years.

In a further step, we examined 35 eyes of 19 patients and 16 healthy control subjects of the "Erlangen Glaucoma Registry." The Erlangen Glaucoma Registry surveils patients with open-angle glaucoma (OAG; open angle glaucoma: primary and secondary due to melanin dispersion and pseudoexfoliation and normal-tension glaucoma [NTG]), suspected glaucoma, or ocular hypertension and healthy control subjects over 15 years. The participants are referred directly by an ophthalmologist or recruited from patients being treated in the hospital because of known or suspected glaucoma. Control subjects are recruited from university staff. Patients are visited annually, they do not have eye diseases other than glaucoma or systemic diseases known to affect the visual system. In this registry, diagnosis of glaucoma is based on visual field test results, glaucomatous optic nerve head damage, and intraocular pressure profile. According to these examinations, patients are classified as having ocular hypertension, preperimetric glaucoma (normal visual field in 30° white-on-white perimetry, plus glaucomatous optic nerve head damage), or perimetric glaucoma (glaucomatous visual field defect in 30° white-on-white perimetry, plus glaucomatous optic nerve head damage). The study adhered to the tenets of the Declaration of Helsinki, and informed consent was obtained from all participants.

Because of possibly altered pupil dynamics, patients with secondary open-angle glaucoma (pigment dispersion syndrome,¹⁷ pseudoexfoliation syndrome [Krist D, et al. *IOVS* 2001;42:ARVO Abstract 2978], or contusion¹⁸) were excluded from the present study, as were patients who had had intraocular surgery¹⁹ or laser-iridotomy. Thus, we included only patients with POAG or NTG. For the same reason, patients with thyroid disorders,²⁰ diabetes,²¹ or neurologic diseases^{22,23} were

FIGURE 2. Parameters of the pupil response as used in the study. Maximum amplitude of the pupil contraction to stimulus offset-onset: 1, $AMPL_{OFF}$ (mm^2); 4, $AMPL_{ON}$ (mm^2). Latency from beginning of stimulus offset-onset to beginning of pupil contraction: 2, T_{OFF1} (ms); 5, T_{ON1} (ms). Latency from beginning of stimulus offset-onset to end of pupil contraction: 3, T_{OFF2} (ms); 6, T_{ON2} (ms). V_{max} of pupil contraction to stimulus offset/onset: 7, $V_{max_{OFF}}$ (mm^2/ms); 9, $V_{max_{ON}}$ (mm^2/ms). Time of V_{max} of pupil contraction to stimulus offset-onset: 8, $T_{V_{max_{OFF}}}$ (ms); 10, $T_{V_{max_{ON}}}$ (ms). Maximum acceleration of pupil contraction to stimulus offset/onset: 11, $A_{max_{OFF}}$ (mm^2/ms^2); 13, $A_{max_{ON}}$ (mm^2/ms^2). Time of maximum acceleration of pupil contraction to stimulus offset/onset: 12, $T_{A_{max_{OFF}}}$ (ms); 14, $T_{A_{max_{ON}}}$ (ms).



excluded from the study. To avoid an influence of daytime variations²⁴ on our results, we performed pupillometry between 11 AM and 2 PM. Visual field testing was performed in all patients and control subjects (model 500; Octopus, program G1; Interzeag, Schlieren, Switzerland). All patients had no ocular diseases other than glaucoma (e.g., myopia >8 D, cataract). Visual acuity was $\geq 16/20$. Glaucomatous changes of the optic nerve were classified according to Jonas et al.²⁵

The subjects were grouped in a healthy control group (intraocular pressure, <21 mm Hg, no visual field defect, normal optic disc) and a glaucoma group (glaucomatous visual field defect, glaucomatous cupping of the optic disc, open chamber angle). On the day of examination, the intraocular pressure had to be below 21 mm Hg.

Statistics

A descriptive approach was chosen, to present the dependence of the pupil response of contrast, luminance, and spatial frequency in three healthy subjects. Responses were plotted for different stimulation modalities (contrast, luminance, and spatial frequency).

Pupillographic measurements of healthy eyes were compared to measurements in the perimetric glaucoma group, using the Mann-Whitney test. When data for both eyes of a subject were available, the eye with more advanced visual field loss was included in the comparison. When the visual field loss was similar in both eyes, one eye was chosen at random.

Differences are detected at the 5% significance level. Differences that were significant only at the 10% level were also reported for exploratory purposes, because the small sample size results in a low power to reject the null hypothesis.

Because pupillographic measurements may be subject to a progression with age, this progression was estimated in the group of healthy eyes. Linear regression relating the measured variables to a subject's age was performed to estimate annual rates of progression. An F-test was used to determine whether the pupillographic variables present a progression at the 5% significance level.

Possible influences of eye color or drug treatment on pupillometric measurements were not examined because of the small sizes of the subgroups.

RESULTS

Spatial frequency, contrast, and intensity influenced pupil reaction to a pattern stimulus. Figure 3A summarizes the results obtained at a contrast of 100%. Plotted were measurements at seven spatial frequencies and eight luminances. It became obvious that with decreasing luminance (Fig. 3B) the pupil responses decreased. A peak of onset and offset response was observed at a luminance of 55 cd/m^2 . Averaged traces indicated that, at all luminance levels tested, the offset component was largest at a low spatial frequency (0.33 cyc/deg), decreased with increasing spatial frequency and finally disappeared at 3.45 or 6.25 cyc/deg and higher. The onset component increased with increasing spatial frequency, reached a maximum at 6.25 cyc/deg and decreased again at 9.2 cyc/deg (Fig. 3C).

Figure 4A shows the influence of contrast on pupil responses. All measurements were obtained at a luminance of 55 cd/m^2 and at three different spatial frequencies (0.33, 1.22, and 6.25 cyc/deg). The higher the contrast, the larger the amplitude and the lower the latency of the offset and onset component. This is exemplarily shown for 0.33 and 6.25 cyc/deg in Figure 4B.

The best pronounced pupil reaction was observed in stimulus conditions as follows: high contrast (100%), high luminance (55 cd/m^2), and low spatial frequency (0.33 cyc/deg) allowed the best recording of the offset response and high contrast (100%), high luminance (55 cd/m^2), and a high spatial frequency (6.25 cyc/deg) allowed the best recording of the onset response. These stimulus conditions were chosen for measurements in the subjects with glaucoma or ocular hypertension and the healthy control group.

In the second part, we examined 16 healthy control eyes and 19 eyes with manifest glaucoma. Table 1 shows the statistical distribution of age, visual field parameters, and different iris colors in the respective groups. Table 2 shows which antiglaucoma eye drops were used.

In the healthy subjects, we did not observe any significant age effect for all parameters in both spatial frequencies. For this

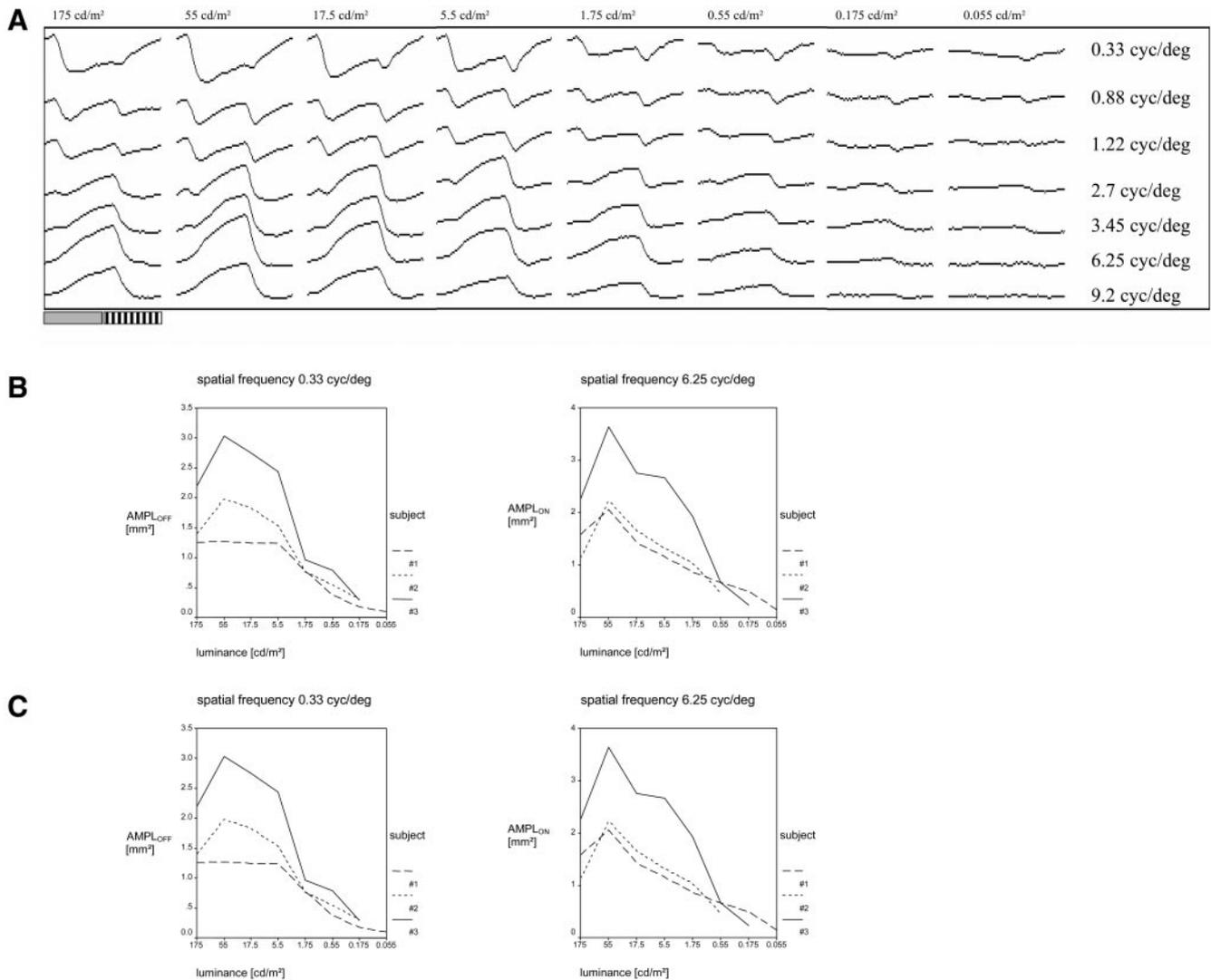


FIGURE 3. Dependence of pupil response of stimulus luminance and spatial frequency. Contrast was 100% in all measurements. **(A)** Traces are averaged pupil reactions of three healthy subjects. **(B)** Dependence of the pupil response of stimulus luminance: amplitude of offset (AMPL_{OFF} at 0.33 cyc/deg) and onset (AMPL_{ON} at 6.25 cyc/deg) component decrease with decreasing stimulus luminance. **(C)** Dependence of the pupil response of spatial frequency of the stimulus: amplitude of offset (AMPL_{OFF}) and onset (AMPL_{ON}) component (luminance of 55 cd/m²). Offset amplitudes were highest at low spatial frequencies, whereas onset amplitudes were highest at high spatial frequencies.

reason, a further age correction was omitted in this preliminary clinical study.

Differences between healthy control eyes and glaucomatous eyes were more often significant at 6.25 cyc/deg than in 0.33 cyc/deg (Table 3). Generally, control eyes had higher amplitudes, velocity, and acceleration of pupil constriction than did glaucomatous eyes. Figure 5 shows the averaged curves of the 16 healthy subjects and the 19 patients with glaucoma.

Comparing control eyes with glaucomatous eyes at 6.25 cyc/deg showed the velocity ($V_{\max_{ON}}$) of the pupil movement to be significantly different between both groups at a 5% level.

In addition, amplitude of the pupil response (AMPL_{ON}) and the maximum acceleration ($A_{\max_{ON}}$) of the pupil movement showed a tendency to be lower in the glaucoma group. The time of the maximum acceleration ($T_{A_{\max_{ON}}}$) of the pupil movement showed a tendency to be higher in the glaucoma group.

Measurements performed with a spatial frequency of 0.33 cyc/deg showed differences between the healthy control and glaucoma groups only in the offset-component. The maximum

velocity of the pupil movement showed a tendency to be higher in the healthy control group ($V_{\max_{OFF}}$) and the time of the highest acceleration of the pupil movement ($T_{A_{\max_{OFF}}}$) was higher in the glaucoma group (10% significance level).

DISCUSSION

Pupil Light Reflex to Patterned Stimuli

This study demonstrated a pupil reflex response to a stripe pattern offset-onset stimulus. A pupil reflex response to this type of stimulus consists of two components, one each to the offset and the onset of the stimulus. It could be demonstrated that, in healthy subjects, the pupil reflex to pattern offset-onset depends on contrast, luminance, and spatial frequency of the stimulus.

The common theory of the pupil reflex cycle is well known. More recently, a new subgroup of cells and its contribution to the pupil light reflex has been characterized. A subset of retinal ganglion cells has been found in rodents, which is intrinsically photosensitive, expresses melanopsin,²⁶⁻³⁰ and has a comple-

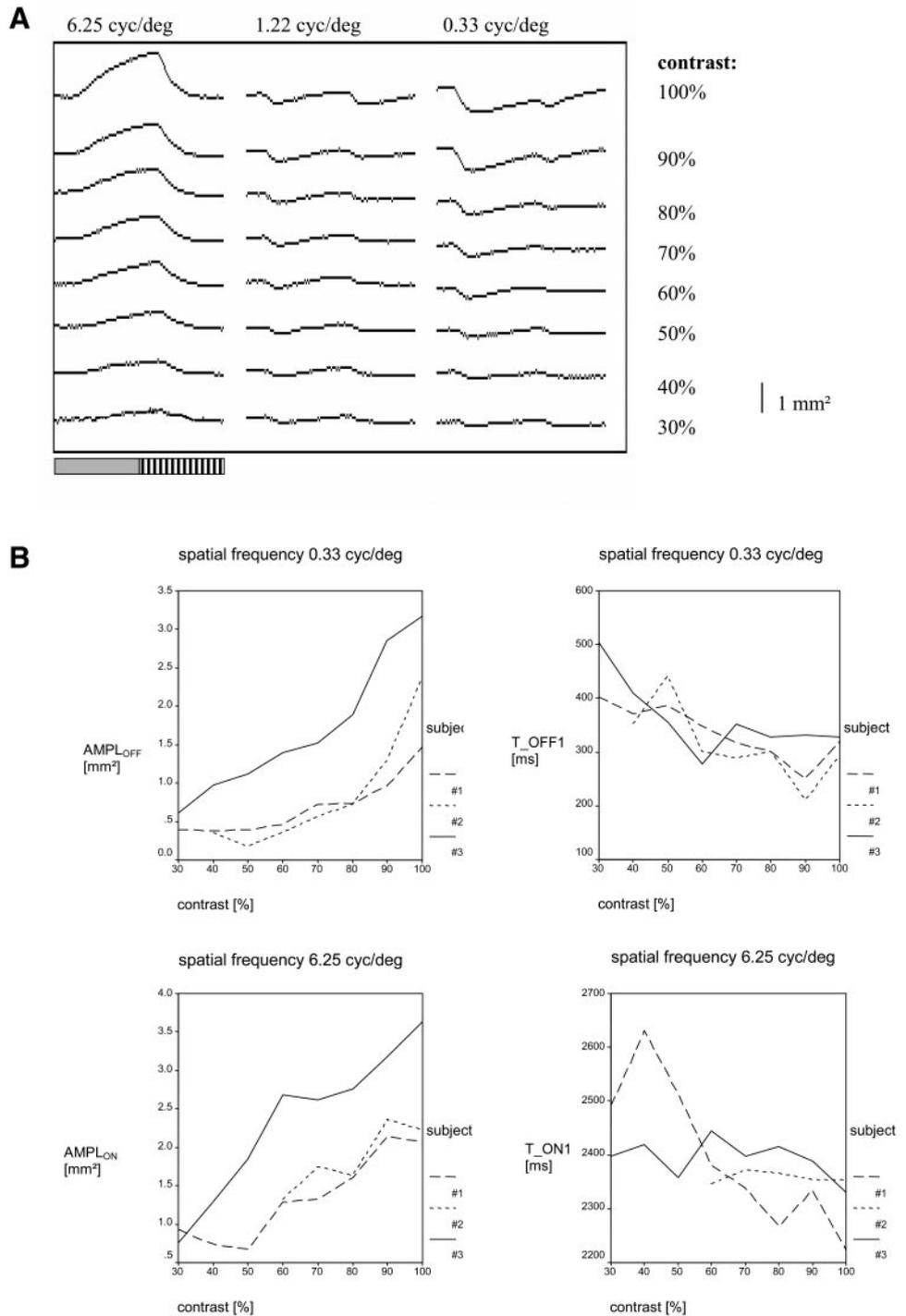


FIGURE 4. Dependence of the pupil response on stimulus contrast. Stimulus luminance was 55 cd/m² in all measurements. **(A)** Traces mean of three healthy subjects. **(B)** Examples shown are amplitude and latency of the offset (AMPL_{OFF}, T_{OFF1} at 0.33 cyc/deg) and onset (AMPL_{ON}, T_{ON1} at 6.25 cyc/deg) components. Amplitudes increased and latencies decreased with increasing stimulus contrast.

mentary role in the pupil light reflex cycle in mice.³¹ The role of these cells in human pupil reflex reaction to patterned stimuli can be hypothesized, but remains unclear at the moment.

It has been demonstrated earlier, that the pupil can respond to patterned stimuli.¹⁻⁴ Our study describes the changes in pupil response coming along with changes of different stimulus conditions such as contrast, luminance, and spatial fre-

TABLE 1. Characteristics of Subjects

Group	Age [years], (range, mean)	MD [dB]	CLV [dB ²]	Iris Color
Control (n = 16)	40-60, 45.5	0.8 ± 0.8	1.6 ± 0.9	7 Blue, 5 green, 1 grey, 3 brown
Glaucoma (n = 19)	21-66, 51.7	5.7 ± 4.3	40.7 ± 36.9	7 Blue, 3 green, 2 grey, 7 brown

TABLE 2. Frequency of Application of Antiglaucoma Eye Drops in the Study Groups

Group	No Therapy	One Drug	More Than 1 Drug	β -Blocker	CAI	α 2-Agonist	PA
Control	16	0	0	0	0	0	0
Glaucoma	3	12	4	3	4	1	12

Data are the number of subjects in each group. CAI, carbonic anhydrase inhibitor; PA, prostaglandin analogue.

quency. Several investigators have reported pupil reflex response to pattern reversal or sine-wave grating stimuli.^{3,5,6,9,10} They come to the conclusion that pattern-evoked pupillary responses depend on the spatial frequency of the pattern^{5,10} and that a pupil response to spatial patterns follows probably another anatomic pathway than a pupil response to luminance changes.³ Our observation goes along with the report by Young and Kennish,¹⁰ who found that the offset component is presented only in spatial frequencies below 2 cyc/deg. The onset components, however, are different in both studies: Whereas Young and Kennish found reproducible onset components at all spatial frequencies tested (0.14–7.32 cyc/deg) with highest amplitudes at 2.98 and 4.62 cyc/deg, we found only a small onset component at 0.33 cyc/deg spatial frequency. From then on, the onset component increased to a maximum at 6.25 cyc/deg and then decreased again. A major difference in our study compared with the work by Young and Kennish is the temporal frequency. Whereas the onset period of the stimulus in the present study was 2000 ms, they chose an onset duration of 6000 ms. An advantage of the latter

approach is the recovery of the pupil diameter to baseline values in most subjects. Nevertheless, as can be seen in the experiments that they performed, an even longer period of stimulus onset would be necessary to guarantee complete recovery in all subjects. In the present study, a shorter duration of the stimulus onset-offset was used, to achieve shorter examination times and thus higher alertness in test subjects, being conscious of the fact that the pupil diameter may not recover completely to baseline when the stimulus alters. Another difference in the present study is that we used a square-wave stimulus, whereas Young and Kennish used a sine-wave stimulus in their work.

Young and Kennish¹⁰ described the dependence of pupil reflex amplitude on stimulus contrast. They generated different pattern stimuli such as pattern onset-offset and pattern reversal on a monitor. As in our study, the first and second peaks of the pupil's response to an onset-offset stimulus increased linearly with increasing contrast, from approximately 20% contrast on. At a stimulus contrast below 30%, we were not able to record reproducible signals, whereas Young and Kennish found a

TABLE 3. Differences between Healthy Eyes and Glaucomatous Eyes in Recordings Performed at 0.33 and 6.25 cyc/deg

	Control Group (Mean \pm SD)	Glaucoma (Mean \pm SD)	<i>P</i>
0.33 cyc/deg: offset component			
Pupil movement			
AMPL _{OFF} (mm ²)	3.06 \pm 1.3	2.50 \pm 1.0	0.281
T _{OFF1} (ms)	263.1 \pm 39.1	284.33 \pm 49.6	0.173
T _{OFF2} (ms)	840.6 \pm 162.5	814.16 \pm 73.6	0.174
Velocity of the pupil movement			
Vmax _{OFF} (mm ² /ms)	52.0 \pm 20.1	40.64 \pm 17.9	0.094
T_Vmax _{OFF} (ms)	469.7 \pm 77.4	489.98 \pm 46.8	0.369
Acceleration of the pupil movement			
Amax _{OFF} (μ m ² /ms ²)	1457.8 \pm 889.8	1070.37 \pm 543.6	0.144
T_Amax _{OFF} (ms)	350.4 \pm 45.3	380.47 \pm 48.4	0.067
0.33 cyc/deg: onset component			
Pupil movement			
AMPL _{ON} (mm ²)	0.73 \pm 0.5	0.86 \pm 0.8	0.585
T _{ON1} (ms)	2334.6 \pm 65.4	2342.94 \pm 82.0	0.747
T _{ON2} (ms)	2662.3 \pm 144.9	2649.76 \pm 102.7	0.779
Velocity of the pupil movement			
Vmax _{ON} (mm ² /ms)	20.92 \pm 13.3	17.98 \pm 12.6	0.665
T_Vmax _{ON} (ms)	2497.8 \pm 97.7	2491.13 \pm 45.6	0.806
Acceleration of the pupil movement			
Amax _{ON} (μ m ² /ms ²)	804.8 \pm 387.2	613.80 \pm 424.5	0.194
T_Amax _{ON} (ms)	2414.6 \pm 80.3	2401.50 \pm 68.6	0.625
6.25 cyc/deg: onset component			
Pupil movement			
AMPL _{ON} (mm ²)	2.93 \pm 1.1	2.09 \pm 1.4	0.060
T _{ON1} (ms)	2283.6 \pm 71.5	2303.4 \pm 96.1	0.497
T _{ON2} (ms)	3043.2 \pm 135.6	2890.8 \pm 571.8	0.274
Velocity of the pupil movement			
Vmax _{ON} (mm ² /ms)	37.16 \pm 15.3	29.61 \pm 18.6	0.001
T_Vmax _{ON} (ms)	2503.0 \pm 41.4	2610.67 \pm 113.3	0.203
Acceleration of the pupil movement			
Amax _{ON} (μ m ² /ms ²)	1104.4 \pm 631.0	746.16 \pm 375.4	0.069
T_Amax _{ON} (ms)	2410.8 \pm 38.1	2467.11 \pm 118.1	0.059

Healthy eyes ($n = 16$); glaucomatous eyes ($n = 19$). Significance level: 5% (bold) and 10% (italic).

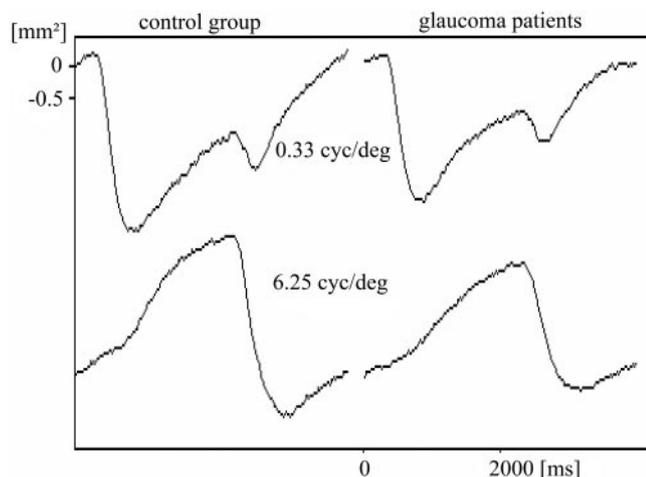


FIGURE 5. Comparison of the averaged curves obtained from 16 control subjects (*left*) and 19 patients with glaucoma (*right*). *Top*: measurements obtained with 0.33 cyc/deg; *bottom*: measurements obtained with 6.25 cyc/deg. A detailed description of the differences between the healthy control group and patients with glaucoma is given in Table 3.

rapid increase in the first and second peak amplitudes with increasing contrast from 0% to 20%.

Decreasing pupil reflexes with decreasing stimulus luminance are expectable, as this is a well-known phenomenon in psychophysics³² and electrophysiology.³³

Pupil Light Reflex and Glaucoma

Our study showed significant differences in pupil dynamics between healthy control subjects and patients with glaucoma. If the stimulus was presented at a spatial frequency of 0.33 cyc/deg, there were significant differences in the time from stimulus offset to the beginning of the pupil reaction (T_{OFF1}) and in the maximum acceleration of pupil reaction to stimulus onset ($A_{max_{ON}}$). If the stimulus was presented at a spatial frequency of 6.25 cyc/deg, differences between the healthy control group and subjects with glaucoma were observed in the amplitude of pupil reaction to stimulus onset ($AMPL_{ON}$), the maximum velocity ($V_{max_{ON}}$) of pupil movement, and the time and amount of maximum acceleration ($T_{Amax_{ON}}$ and $A_{max_{ON}}$) of pupil movement.

Our results are based on pupil responses to patterned stimuli and thus are difficult to compare with results of other studies. Nevertheless, there are some parallels as to a reduced amplitude and prolonged latencies of the pupil reflex in subjects with glaucoma.

Several studies have been performed in the past examining the pupil light reflex in ocular hypertension and glaucoma and thus confirmed its importance in clinical examination. Previous studies by our group have already demonstrated altered pupil reaction in patients with glaucoma to light flash stimuli (Krist D, et al. *IOVS* 2001;42:ARVO Abstract 2978). A reduced-light reflex amplitude may be found in acute POAG.³⁴ A prolonged pupil cycle time may be observed in primary closed-angle glaucoma³⁵ and in open-angle glaucoma.³⁶ An afferent pupillary defect is detectable in patients with glaucoma^{37,38} and correlates with the difference between the eyes in rim area and cup-to-disc ratio.³⁹

Pupil perimetry is another technique applied in glaucoma diagnosis. Its capability of detecting glaucomatous visual field defects is a subject of controversy. Whereas there is a report of good correlation between glaucomatous visual field defects and reduced pupil movement,⁴⁰ another did not find such re-

sults.⁴¹ Multifocal pupillometry has been performed recently,^{42,43} showing a good agreement between visual field defect and pupil movement.

Factors Influencing the Pupil Light Reflex

Among possible factors influencing pupil size and pupil light reflex are age, iris color, retinal illumination, alertness, local antiglaucoma therapy, and stimulus characteristics.

It is well known that pupil size decreases with increasing age.⁴⁴ Also pupil dynamics to a flash stimulus change with increasing age.⁴⁵ The latency time is prolonged and the ratio of the velocity of constriction against the velocity of dilatation correlates negatively with age. In our relatively small group of healthy control subjects we did not observe any significant age dependence of the parameters of the pupil reflex.

Differences in pupil dynamics relative to iris color have been observed.⁴⁶ These differences between brown and blue irides were observed in constriction amplitude, redilatation velocity, and constriction velocity, whereas initial pupil size and latency time were independent of iris color. This was also confirmed by an earlier study,⁴⁴ which found pupil size to be independent of gender, refractive error, or iris color. In the present study, 10 of 35 eyes (28.3%) examined had brown irides.

The state of alertness influences pupil size and pupil dynamics.^{47,48} This effect can be disregarded in our study, as all participants were awake and all were examined between 10 AM and 2 PM.

The pupillary light reflex is furthermore influenced by characteristics of the light stimulus (light intensity, duration, area, perimetric location, temporal, and spatial frequency of the stimulus and motion) and the stimulated eye (state of light adaptation and spectral sensitivity of the stimulated photoreceptors).⁴⁹ Light stimulus and light adaptation of the stimulated eye were kept constant for all subjects.

The influence of common antiglaucoma eye drops on pupil movement has been studied by various authors. The influence of most antiglaucoma eye drops on pupil width and pupil movement is controversial (brimonidine⁵⁰⁻⁵³, latanoprost,^{51,54} and timolol^{55,56}). The topical carbonic anhydrase inhibitor dorzolamide does not seem to influence amplitude and latency of pupil contraction in humans.⁵⁷ These experiments have been performed with light-stimuli rather than pattern-reversal stimuli and/or mainly describe changes in pupil width rather than in pupil dynamics. Only latanoprost is known to alter latency of the pupil reflex reaction to a light stimulus,⁵⁶ whereas other antiglaucoma drugs mainly influence pupil diameter.

The sample size in the present study is too small to account for each of the factors just described. Nevertheless, the present study demonstrates the feasibility of the test in clinical routine. As there are many factors influencing the pupil reflex, several detailed studies are needed to examine the effect of these factors on the pupil reflex response to patterned stimuli. As shown earlier, several differences between the healthy control and glaucoma groups were demonstrated in the present study. Recently, as mentioned earlier, a new subset of ganglion cells involved in the pupil reflex has been described, the melanopsin cells.³¹ At the moment it remains unclear, whether these cells are involved in creating a pupil reflex response to patterned stimuli and whether they are damaged in glaucoma. In our opinion, pupillography, as described herein, together with anatomic examinations may give important insight into this topic.

CONCLUSIONS

In this study, we describe the human pupil response to a patterned stimulus (onset-offset, stripe pattern). Spatial frequency, luminance, and contrast play a role in the course of the pupil response of healthy subjects. In a preliminary clinical study, we demonstrated significant differences between pupil responses of healthy persons and those with glaucoma, but for a better understanding of the influence of factors such as age, local antiglaucoma medication, and eye disease, such as pseudoexfoliation and melanin dispersion, further investigations must be performed.

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