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Pupillometric characteristics in patients with choroidal neovascularization due to age-related macular degeneration

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PURPOSE. *To study the pupillary light reflex in patients with choroidal neovascularization due to age-related macular degeneration (AMD).*
METHODS. *The study included 15 patients with AMD and 15 control subjects. A full recording of the pupil's reaction to light was registered and the following eight parameters were measured and reported: baseline pupil radius (R1), latency (T1), minimum pupil radius (R2), amplitude (AMP), maximum constriction velocity (VCmax), maximum constriction acceleration (ACmax), time for maximum velocity (T2), and time for maximum constriction (T3).*
RESULTS. *All variables measured presented alterations in the AMD group and a number of them were significantly reduced in the AMD group.*
CONCLUSIONS. *The presence of neovascular AMD significantly affects the pupil's response to light stimulus when compared to normal subjects. (Eur J Ophthalmol 2009; 19: 254-62)*

KEY WORDS. *Pupillometry, Age-related macular degeneration*

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INTRODUCTION

The pupillary light reflex (PLR) is a valuable objective test used to evaluate visual function, providing information about the integrity of the afferent and efferent pathways. The pupillary reflex itself depends on several parameters besides the pupil itself and the stimulus' characteristics, i.e., the photoreceptors, the ganglion cell axon, the optic nerve and the chiasm, the optic tract, the superior colliculus brachium, the pretectal area, the interconnection neurons in the IIIrd nerve nucleus, the efferent parasympathetic pathways accompanying the IIIrd nerve, and the efferent sympathetic pathways from the hypothalamus to the dilator muscle (1).

The majority of previous published reports (1-5) on pupillary movements studied the central nervous system (CNS), the efferent sympathetic pathways, and the smooth muscles of the iris (sphincter and dilator). The pupil size affects the level of visual acuity by increasing the effects of diffraction when pupil is constricted. The size of the pupil also indirectly affects the visual acuity by reducing the amount of light entering the eye when the light stimulus is intense and conversely increasing light capture under dim lighting conditions.

Previous studies (6-22) have shown that pupil latency and cycling time can be delayed in patients with afferent diseases such as demyelinating diseases (15), Leber optic neuropathy (18), amblyopia and optic atrophy (19-20), and also in efferent pupil disorders that affect the autonomic innervation of the iris such as diabetes.

Patients with unilateral or asymmetric retinal or optic nerve damage will show contraction of both pupils when the light is moved towards the unaffected eye, and they will both dilate when the light is shifted to the affected eye. When bilateral impairment of the retina or optic nerve is present, stimulation of either eye (or of both of them simultaneously) will elicit subnormal contraction of low intensity.

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Age-related macular degeneration (AMD) is the leading cause of irreversible blindness (23). It is estimated that AMD occurs in 1.4% of the population over the age of 40 with highest prevalence occurring in subjects from 75–84 years of age (24). AMD is clinically classified into two major groups: the nonexudative (dry) and the exudative (wet) form. The gradual disappearance of the retina pigment epithelium (RPE) in the dry form results in patches of chorioretinal atrophy without any visual function. In the other form (wet), damage arises from the subretinal fluid/intraretinal fluid, blood, or destruction of photoreceptors and RPE by fibrous or fibrovascular tissue (25-27). Since the retinal rods and cones are the initial receptors of the pupillary light reflex (PLR), any kind of retinal or optic lesion may reduce the afferent pupillary impulses and cause low intensity reflexes. This reflection of retinal or optic nerve lesion to the PLR has not yet been documented.

The recent development of an infrared pupillometer with computer-assisted analysis (28) has allowed a detailed evaluation of the pupillary light responses since the previous methods of pupillary evaluation were very limited. This method is a noninvasive, nondependent on epithelial absorption of a pharmaceutical agent method. It provides a direct evaluation of the eye under light and dark circumstances and can obtain a recording of the pupillary response to different stimuli in light and dark.

The aim of this study was to evaluate objectively the pupillary light reflex in patients with neovascular AMD and to compare it to the pupillary light reflex of normal subjects.

PATIENTS AND METHODS

A total of 30 subjects were included in this study: 15 patients with neovascular AMD and 15 subjects with healthy eyes matching sex and age. Both patients and controls were physically healthy and did not have any neurologic disorders. All subjects underwent a complete ophthalmologic evaluation and photography of the fundus as well as fluorescein angiography (FA), pattern reversal visual evoked potential (PR-VEP), and electroretinogram (ERG) for the group of patients with AMD; all results were compatible with their clinical status. None of the patients had undergone previous cataract surgery, or had history of any disease that might result in anterior synechiae (e.g., uveitis) or could otherwise affect their pupillary response.

Patients with AMD had a choroidal neovascular membrane or a disciform scar at the macular region >2 disc diameters in one eye and no similar lesion in the fellow eye. Visual acuity of the affected eye was less than 20/200 (logMAR < 1) and varied from counting fingers (CF) to hand motion (HM). Because patients with AMD tend to present with prodromal AMD lesions in the fellow eye, we decided to have a separate group of patients without any signs of AMD lesions in both eyes, to serve as a control group. Control subjects did not have any eye disorder and had a corrected visual acuity of 20/20 (logMAR 0). The best-corrected visual acuity was determined using a Snellen chart. Visual acuity was analyzed for study purposes by means of the logMAR score. The logMAR score is derived by taking the logarithm (base 10) of the fraction of the Snellen visual acuity. A score of 0.0 is equivalent to a visual acuity of 20/20 on the Snellen acuity chart, whereas a score of +1.0 is equivalent to 20/200. The study was conducted in the Laboratory of Clinical Neurophysiology of the Aristotle University of Thessaloniki, of AHEPA University Hospital. All participants provided written informed consent and all experiments were approved by the Ethical Committee of the AHEPA University Hospital based on the Helsinki Declaration.

Pupillographic device (30-32)

The system for recording PLR is monocular and works fully automated. It consists of the following items:

I. A CCD high-speed digital camera with maximum responsivity in the red and infrared region of the spectrum. The camera is capable of taking a maximum of 262 frames per second. The actual speed of the camera is controlled by the software developed for this purpose. Because of the corneal curvature and in order to avoid errors due to optical distortion, the camera is set normal to the axis of the eye and at a distance of 30 cm away, so that the image of the pupil is symmetrical.

II. A computer and the associated sampling cards.

III. Two independent light sources:

a) An infrared light source which illuminates the face of the person, consisting of an array of 32 LED at 820 nm maximum, switched on permanently throughout the measurement. The infrared light used is considered harmless to the eye apparatus as it is diffuse, emitted by two rather large sources located nearby and is of low intensity. Furthermore, it does not influence the results, as the human retina is known to be insensitive to moderate intensities of infrared light.

b) A clinical photic stimulator (SLE), made by BioLogic System Corporation UK. A diffuse flash light of 20 msec duration and 24.6 candelas/m^2 intensity, which is produced by a light bulb through the discharge of a capacitor, is shed in the whole of the visual field predominantly affecting the posterior pole of the retina.

IV. A traversing mechanism. The whole instrument is based on an optical examination table with a head rest fixing the position of the head on one side. On top of the table the camera is fixed on a mechanism which is able to move in three, x-y-z, directions. The camera can also be rotated in the x-y and x-z planes.

V. An image processing analysis system which calculates the parameters of the PLR in real-time. The recording of these parameters ceases automatically after 3.5 seconds from the application of flash light.

Experimental conditions (30-32)

After a full clinical examination, the experimental conditions were explained to each subject in detail and the following procedure was applied. Each subject remained for 2 minutes in complete darkness so that the maximum darkness diameter could be approached and the pupillary oscillations-fatigue waves that appear later could be avoided. Each subject was instructed to focus on an infrared light on the same axis as the camera, and at a distance of 1.5 m. Following the dark adaptation period five rectangular light flashes, with a 30 sec interval, were administered. In the 30 sec interval a full record of the pupil's reaction radius and center, as a function of time, was recorded and then analyzed on line. In both groups blindly, it was decided manually whether the recording should be deleted or saved (if artefact free).

The calculated parameters were as follows (Fig. 1):

1. Baseline pupil radius after a 2-min dark adaptation (R1)
2. Latency for the onset of constriction (T1); T1 is defined as the time of maximum constriction acceleration
3. Amplitude of reaction (AMP or R1-R2, where R2 is the maximum constriction radius)
4. Maximum constriction velocity (VCmax)
5. Maximum constriction acceleration (ACmax)
6. Latency of maximum constriction (T2); this is defined as the time when the constriction velocity is zero
7. Maximum constriction radius (R2)
8. Time for maximum constriction (T3)

The measurement and recording necessary to obtain the above parameters covered the changes to the size of the pupil for a period of 3.5 sec from the application of the light flash. Latency is defined as the time of pupil's maximum acceleration, while time of maximum miosis is the time of zero constriction velocity.

The use of radius instead of the diameter seems more reliable and appropriate. It can be used even when the upper lid covers part of the pupil; it is also very easy to

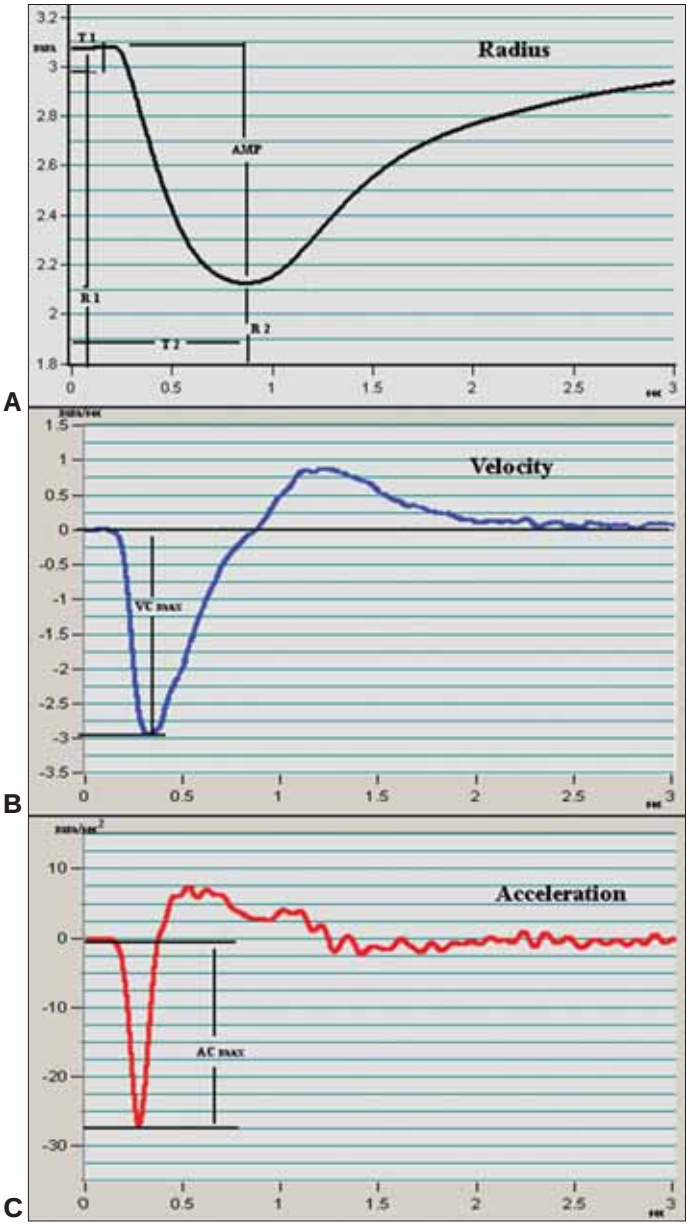

Fig. 1 - *Basic parameters measured: baseline pupil radius (R1), maximum constriction radius (R2), latency for the onset of constriction (T1), latency of maximum constriction (T2), amplitude (AMP)* **(A)**, *maximum constriction velocity (VCmax)* **(B)**, *maximum constriction acceleration (ACmax)* **(C)**.

translate all data using radius to diameter, since all values are multiplied by two (×2).

Statistical analysis

Due to the nature of the dependent variables (continuous) parametric methods were used for the analysis of the data. In Tables I and II, descriptive statistics are presented for the two groups for all the dependent variables.
Table III presents the p values of the Student *t*-test for all measured variables. The statistical analysis included the calculation of means and standard deviations for each pupillographic variable. All the statistical analyses were calculated according to the measurements of five artefact-free (averaged) pupil light response curves of the right eye as no statistical difference was found between the two eyes (p>0.89).

In addition, a correlation of all variables in both groups was made. The results of this correlation are presented in Table IV.

RESULTS

Tables I and II show all measured variables for normal subjects and patients with neovascular AMD (mean value and standard deviations). The most important variables presenting significant alterations were those of ACmax, VCmax, and AMP.
As far as ACmax is concerned, the mean value in normal control subjects was –10.171±2.956 sec^2, whereas in patients with neovascular AMD it was significantly (p<0.05) reduced to –21.269±4.185 sec^2.
Furthermore, VCmax also showed a significant reduction

TABLE I - DESCRIPTIVE STATISTICS: PATIENTS

	No.	Minimum	Maximum	Mean	SD
T1 (msec)	15	0.24760	0.29000	0.2588696	0.01254545
R1 (mm)	15	1.09704	2.29721	1.7640603	0.28110468
R2 (mm)	15	0.79281	1.87514	1.5270535	0.23384681
T2 (msec)	15	0.63462	0.86962	0.6847397	0.05821576
VCmax (mm/sec)	15	–1.95916	–0.57282	–0.9757993	0.38910799
T3 (msec)	15	0.28846	0.39231	0.3412244	0.02930943
ACmax (sec^2)	15	–17.71868	–4.94656	–10.1716539	2.95627017
AMP (mm)	15	–0.64811	–0.05976	–0.2370068	0.14050569
Valid N (listwise)	15				

Descriptive statistics (minimum, maximum, mean, and standard deviations) of the pupillary light reflex variables in the age-related macular degeneration group.
T1 = latency for the onset of constriction; R1 = baseline pupil radius after a 2-min dark adaptation; R2 = maximum constriction radius; T2 = latency of maximum constriction; VCmax = maximum constriction velocity; T3 = time for maximum constriction; ACmax = maximum constriction acceleration; AMP = amplitude of reaction.

TABLE II - DESCRIPTIVE STATISTICS: NORMAL CONTROLS

	No.	Minimum	Maximum	Mean	SD
T1 (msec)	15	0.17308	0.24615	0.2171795	0.02268435
R1 (mm)	15	1.31191	2.96815	2.0647372	0.61109568
R2 (mm)	15	0.92469	2.21092	1.3981653	0.44474363
T2 (msec)	15	0.69231	0.97308	0.8137692	0.09091973
VCmax (mm/sec)	15	–2.82933	–1.47773	–2.2428559	0.43148211
T3 (msec)	15	0.28846	0.36635	0.3188333	0.02590791
ACmax (sec^2)	15	–27.41112	–14.02077	–21.2698184	4.18538043
AMP (mm)	15	–1.02417	0.97357	–0.2246970	0.68982354
Valid N (listwise)	15				

Descriptive statistics (minimum, maximum, mean, and standard deviations) of the pupillary light reflex variables in the group of normal controls.
T1 = latency for the onset of constriction; R1 = baseline pupil radius after a 2-min dark adaptation; R2 = maximum constriction radius; T2 = latency of maximum constriction; VCmax = maximum constriction velocity; T3 = time for maximum constriction; ACmax = maximum constriction acceleration; AMP = amplitude of reaction.

TABLE III - GROUP STATISTICS

	Patients/controls*	No.	Mean	SD
T1 (msec)	1	15	0.2588696	0.01254545
	2	15	0.2171795	0.02268435
R1 (mm)	1	15	1.7640603	0.28110468
	2	15	2.0647372	0.61109568
R2 (mm)	1	15	1.5270535	0.23384681
	2	15	1.3981653	0.44474363
T2 (msec)	1	15	0.6847397	0.05821576
	2	15	0.8137692	0.09091973
VCmax (mm/sec)	1	15	–0.9757993	0.38910799
	2	15	–2.2428559	0.43148211
T3 (msec)	1	15	0.3412244	0.02930943
	2	15	0.3188333	0.02590791
ACmax(sec^2)	1	15	–10.1716539	2.95627017
	2	15	–21.2698184	4.18538043
AMP (mm)	1	15	–0.2370068	0.14050569
	2	15	–0.2246970	0.68982354

p Values for all measured variables in both groups (Student t-test).

*Patients/controls: 1/2.

T1 = latency for the onset of constriction; R1 = baseline pupil radius after a 2-min dark adaptation; R2 = maximum constriction radius; T2 = latency of maximum constriction; VCmax = maximum constriction velocity; T3 = time for maximum constriction; ACmax = maximum constriction acceleration; AMP = amplitude of reaction.

TABLE IV - CORRELATIONS

		T1	R1	R2	T2	VCmax	T3	ACmax	AMP
T1 (msec)	Pearson correlation	1	–0.482*	0.017	–0.772*	0.834*	0.447†	0.888*	0.185
	Significance (two-tailed)	—	0.007	0.929	0.000	0.000	0.013	0.000	0.328
	N	30	30	30	30	30	30	30	30
R1 (mm)	Pearson correlation	–0.482*	1	0.815*	0.779*	–0.537*	0.034	–0.539*	0.259
	Significance (two-tailed)	0.007	—	0.000	0.000	0.002	0.860	0.002	0.168
	N	30	30	30	30	30	30	30	30
R2 (mm)	Pearson correlation	0.017	0.815*	1	0.357	–0.007	0.342	0.006	0.472*
	Significance (two-tailed)	0.929	0.000	—	0.053	0.970	0.064	0.977	0.008
	N	30	30	30	30	30	30	30	30
T2 (msec)	Pearson correlation	–0.772*	0.779*	0.357	1	–0.799*	–0.144	–0.803*	0.126
	Significance (two-tailed)	0.000	0.000	0.053	—	0.000	0.447	0.000	0.506
	N	30	30	30	30	30	30	30	30
VCmax (mm/sec)	Pearson correlation	0.834*	–0.537*	–0.007	–0.799*	1	0.485*	0.975*	0.113
	Significance (two-tailed)	0.000	0.002	0.970	0.000	—	0.007	0.000	0.551
	N	30	30	30	30	30	30	30	30
T3 (msec)	Pearson correlation	0.447†	0.034	0.342	–0.144	0.485*	1	0.488*	0.391†
	Significance (two-tailed)	0.013	0.860	0.064	0.447	0.007	—	0.006	0.033
	N	30	30	30	30	30	30	30	30
ACmax (sec^2)	Pearson correlation	0.888*	–0.539*	0.006	–0.803*	0.975*	0.488*	1	0.167
	Significance (two-tailed)	0.000	0.002	0.977	0.000	0.000	0.006	—	0.377
	N	30	30	30	30	30	30	30	30
AMP (mm)	Pearson correlation	0.185	0.259	0.472*	0.126	0.113	0.391†	0.167	1
	Significance (two-tailed)	0.328	0.168	0.008	0.506	0.551	0.033	0.377	—
	N	30	30	30	30	30	30	30	30

Correlations of all eight pupillary light reflex variables between the two groups.

*Correlation is significant at the 0.01 level (two-tailed).

†Correlation is significant at the 0.05 level (two-tailed).

T1 = latency for the onset of constriction; R1 = baseline pupil radius after a 2-min dark adaptation; R2 = maximum constriction radius; T2 = latency of maximum constriction; VCmax = maximum constriction velocity; T3 = time for maximum constriction; ACmax = maximum constriction acceleration; AMP = amplitude of reaction.

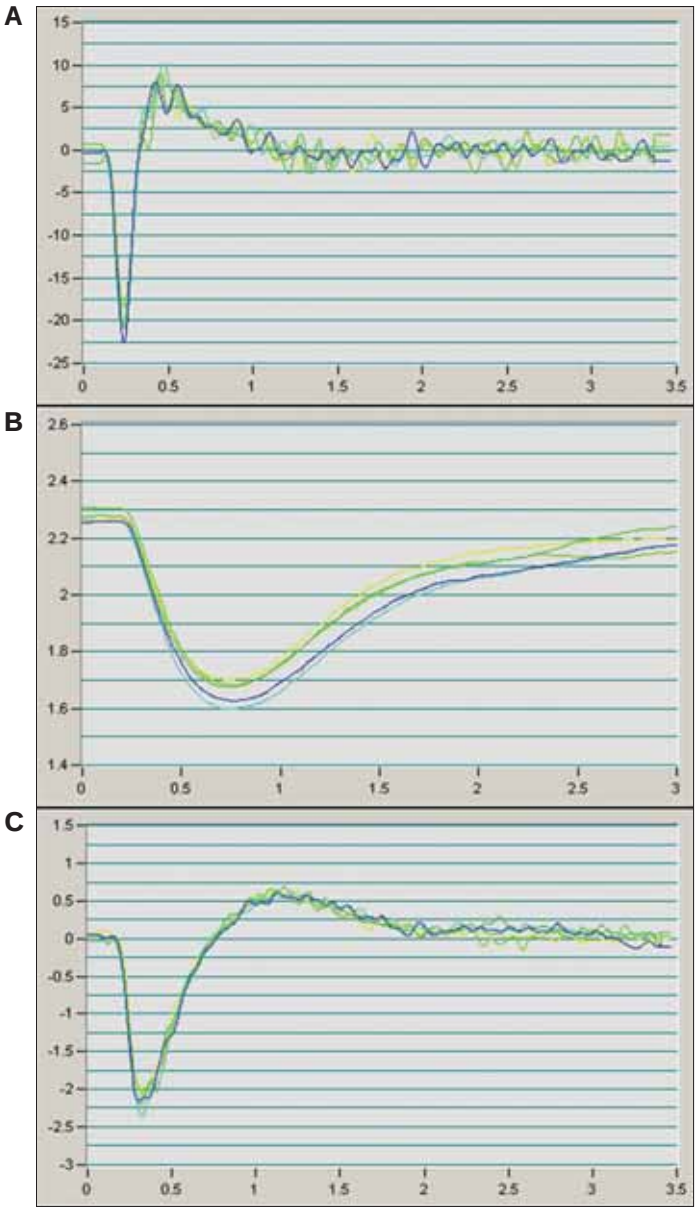

Fig. 2 - *Acceleration (ACmax)* **(A)**, *baseline radius (R1)* **(B)**, *and velocity (VCmax)* **(C)** *of an 83-year-old normal subject.*

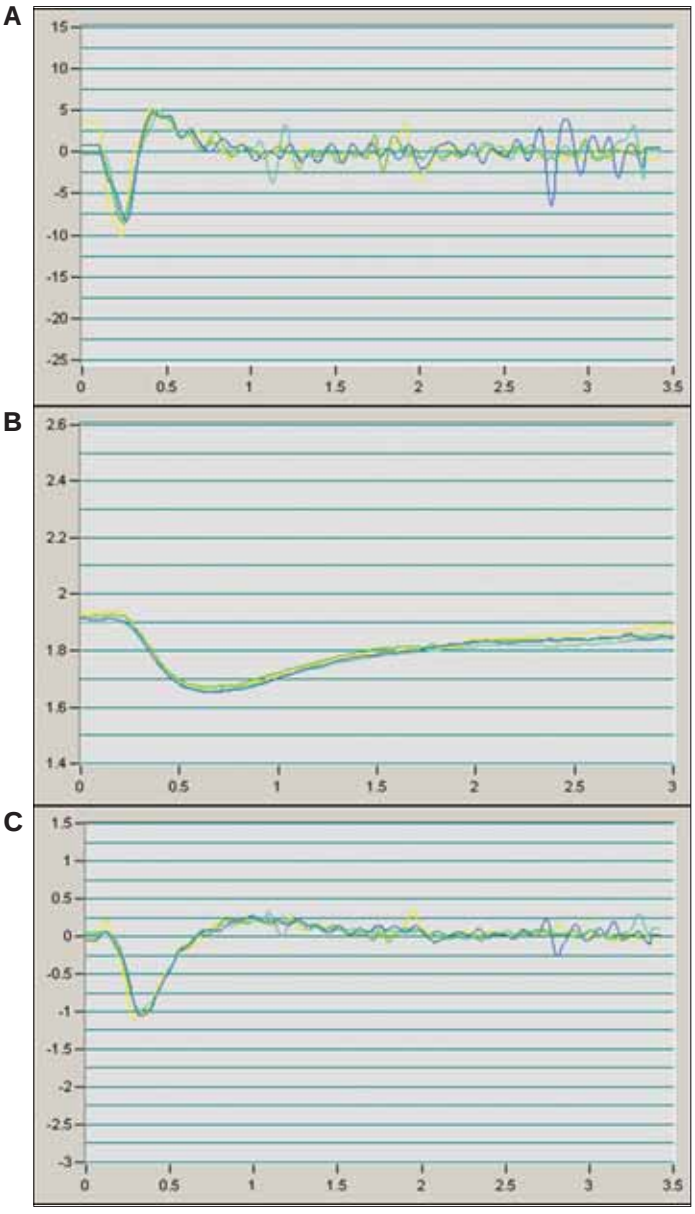

Fig. 3 - *Acceleration (ACmax)* **(A)**, *baseline radius (R1)* **(B)**, *and velocity (VCmax)* **(C)** *of an 83-year-old patient with age-related macular degeneration.*

in patients with neovascular AMD (–2.242±0.431 mm/sec) when compared to those of the normal subjects (–0.975±0.389 mm/sec) ($p<0.05$).

Finally, for AMP, the reduction in patients with neovascular AMD was also significant when compared to the control group (–0.237±0.14 mm and 0.224±0.689 mm, respectively) ($p<0.05$).

In addition to the abovementioned results, the remaining variables also showed variations—thought not statistically significant—when compared to the control group. Namely, T1 was less markedly prolonged in patients with neovascular AMD (0.258±0.125 msec) compared to controls (0.22±0.004 msec). Also, R1 in the neovascular AMD group (1.764±0.281 mm) was below the mean value for normal subjects (2.064±0.611 mm) and T2 showed decrease in patients with neovascular AMD (0.684±0.582

msec) when compared to controls (0.813±0.909 msec).
Figures 2 and 3 present the schematic representations of ACmax, R1, and VCmax in a normal subject and a patient with neovascular AMD matching age and sex.
The ratio of minimal to initial radius showed a significant increase in neovascular AMD as shown from the values in Table III. The ratio of final to initial radius of the pupil is also significantly increased compared to controls.
There was no correlation present between the initial pupil size and VCmax and ACmax (Tab. IV).

DISCUSSION

Pupil size evaluation is a standard procedure in every neuro-ophthalmologic examination and diagnosis is frequently based on the examiner's personal evaluation. Video pupillometry is a more objective measurement of pupil size with an infrared technology that can determine the size of the pupil with a deviation of mm^{-2} (28).
We have previously shown (29-31) that infrared pupillometry is a reliable method of pupil measurement. R1, AMP, R2, ACmax, and VCmax are the most important variables characterizing the pupillary light reflex that can be measured and evaluated with the use of infrared pupillometry.
Patients referred for any reason for a neuro-ophthalmologic examination or an evaluation of their pupil size and reaction may not always be healthy normal subjects without ophthalmologic disease, and this is where the above evaluation becomes more complicated. One of the most frequent posterior segment diagnoses over the age of 50 is AMD.
AMD is the leading cause of blindness in the Western world, and is expected to affect 3 million people in 2020 in the United States alone (32). Patients with AMD have degeneration of the retinal pigment epithelium, Bruch membrane, and choriocapillaris. Like other patients with degenerative diseases of the macula, destruction or defects in cone adaptation can be clinically significant. Their visual acuity can vary from lightly reduced to heavily impaired (CF or even light perception).
The pupil of an eye blinded from retinal or optic nerve diseases will fail to constrict consensually when the contralateral healthy eye is stimulated. The blind eye is regarded to show a relative afferent pupillary defect as the pupillomotor stimulus reaches the brain from the blind eye. When a unilateral macular disease is present and the central scotoma includes the blind spot, a relative afferent defect is present.
The results of our study report that all measured variables showed alterations in patients with neovascular AMD and a statistically significant reduction in some of those values. More specifically, we report that ACmax, VCmax, and AMP were markedly reduced in patients with neovascular AMD when compared to controls. The latter shows that pupil reaction to stimulus varies in patients with neovascular AMD, showing an important reduction in acceleration, velocity, and amplitude.
The ratio of minimal to initial radius showed a significant increase in neovascular AMD as shown from the values in Table III. The ratio of final to initial radius of the pupil is also significantly increased compared to controls. These results imply that the pupil of patients with AMD has smaller size variations than in normal subjects and can be easily tracked when the test is performed.
Our study also showed that when all variables were correlated between the two groups, acceleration and velocity were independent of the baseline pupil radius. This finding has clinical implications: the initial pupil size is relatively smaller in older age and that could complicate the evaluation of velocity and acceleration. Previous studies have reported evaluation of the pupil reflex as a way to study any possible deficit that may occur in pupil mobility in alcoholism (33), diabetes (19), Down syndrome (34), depression (35), generalized anxiety disorder (36), mental retardation (34), Alzheimer disease (6-12), Parkinson disease (13), multiple sclerosis (14, 16), cognitive and emotional process (37), fatigue (38), drug abuse (39-41), and to elucidate the mode of action of specific antidepressants (42, 43). Other studies have evaluated the pupil's cycle time and oscillations in patients with AIDS (44).
To our knowledge, there is no other study on pupillometric evaluation of the pupil's light reflex in patients with AMD. Velocity, amplitude, and the duration of the pupillary light reflex depends on the kind of light used as stimulus: its intensity, duration, color, area of retinal distribution, and waveform. These characteristics of the light determine the output of the retinal receptors that is the summed activity of the rods and cones. These two receptor systems furnish afferent impulses for the pupil, as they do for vision. For this reason, visual sensation and pupillary movements have many parallel features.
The current study supports the hypothesis that the presence of macular degeneration in patients with AMD affects the pupil's response to light stimulus. It is evident from our study that the pupil has a reduced activity not only in terms of radius size variation but mainly in

acceleration and velocity. What we believe to be important in our study is that the pupillary reflex in the current study group is only influenced by the retina layer status since the integrity of the neuroanatomic pathway is granted. Based on the nature of AMD disease, we have to respect the hypothesis that our results are affected only by the retinal and choroidal disease, and not by the neuroanatomic pathway. Although there is a definite cone predominance in the macular area, we cannot attribute any possible explanation or link of this anatomic predominance to our results. The exact mechanism by which the affected macula influences the pupil reflex and whether an affected pupil reflex in a normal subject could eventually prognose a macular disease is still under investigation.

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