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Cholinergic deficiency in Alzheimer's and Parkinson's disease: Evaluation with pupillometry

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ABSTRACT

The aim of the study was to evaluate the cholinergic deficiency in Alzheimer's (AD) and Parkinson's disease (PD). For this purpose, pupil size changes and mobility were assessed using a fast-video pupillometer (263 frames/s). Twenty-three (23) patients with probable AD and twenty-two (22) patients with PD (eleven with cognitive impairment and eleven without) entered the study.

A full record of the pupil's reaction to light was registered. From this data ten (10) parameters were measured and reported. Comparison of those parameters in both group of subjects followed.

Patients with probable AD had abnormal pupillary function compared to healthy ageing. All the Pupil Light Reflex (PLR) variables significantly differed between the two groups ($p<0.005$) except the Baseline Pupil Diameter after 2-min dark adaptation ($D1$) and the Minimum Pupil Diameter ($D2$). Maximum Constriction Acceleration (ACmax) was the best predictor in classifying a subject as normal or as an AD with a perfect classification ability (AUC = 1, $p<0.001$).

ACmax and Maximum Constriction Velocity (VCmax) were significantly lower in PD patients without and with coexisting cognitive impairment compared to normal subjects ($p<0.001$). Patients with cognitive impairment had significantly lower levels of ACmax, VCmax and amplitude (AMP = $D1$–$D2$) than patients with no cognitive deficits. ACmax and secondarily VCmax were the best predictors in classifying a subject as normal or as a PD patient with or without cognitive impairment.

Cognitive and memory impairment, which reflects a cholinergic deficit, may be a crucial pathogenetic factor for the decrease in the aforementioned pupillometric parameters. VCmax and ACmax can be considered as the most sensitive indicators of this cholinergic deficiency.

1. Introduction

There is substantial evidence reporting a profound reduction of cortical choline acetyltransferase and cholinergic neuronal loss in patients with Alzheimer disease (AD) (Davies and Maloney, 1976; Bowen et al., 1976).This evidence implicates that cholinergic hypofunction is considered as a significant component of this disorder.

In addition, Dementia in Parkinson disease (PD) as studied with PET, is found to be common, thus, not sufficiently understood in terms of pathophysiology (Mahler and Cummings, 1990). Significant loss of cholinergic forebrain neurons has also been reported in PD-affected brains (Whitehouse et al., 1983; Candy et al., 1983). Findings of greater forebrain neuronal loss in patients with PD than patients with AD, led Arendt et al. (1983) to propose that the Central Cholinergic System (CSS) deficits may be at least as prominent in PD as in AD.

The Central Cholinergic System (CSS) is composed of closely aligned neurons that cover a wide area within the brain, from the forebrain to the spinal motor nuclei. These neurons do not function independently, but are interconnected and affect each other. The pathophysiology of many neuropsychiatric disorders is partly attributed to such a deficit. However, due to the complexity of the CSS and the broad range of muscarinic and nicotinic receptors that can be identified in the cortical and subcortical areas, the evaluation of the CSS is even nowadays extremely difficult.

Pupillometry is a fast, low cost, non-invasive technique that has been applied in the monitoring of the CSS in diseases, such as Parkinson's Disease, Alzheimer's Disease, Myasthenia Gravis, Schizophrenia and Depression. The pupillary constriction and the redilation process are governed by the action of the Edinger–Westphal nucleus (EW) and the Locus Coeruleus (LC) through their projections on the ciliary ganglion and the ciliospinal center of Budge–Waller respectively.

The main reason why the Pupillary Light Reflex (PLR) is used for our purpose is that Acetylcholine (ACh) is the main neurotransmitter that participates in this projection. More specifically, ACh is the

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neurotransmitter involved in the ciliary ganglion and the sphincter muscle (Guyton and Hall, 1996). The dilator's muscle reciprocal inhibition is accomplished through ACh (Loewnfeld, 1999). Additionally, the visual cortical areas that participate in the PLR contain ACh (Kardon, 1998). Likewise, the Edinger–Westphal (EW) nucleus is cholinergic in nature (Granholm et al., 2003). Finally, according to Loewnfeld (1999), the sympathetic contribution to the PLR is minimal, proving the latter's extreme sensitivity to the measurement of cholinergic changes within the CNS. Based on these facts, the recording of the PLR and the analysis of its parameters has so far revealed a significant cholinergic deficit in several disorders whose pathology lies within the CNS, such as Parkinson's disease (Granholm et al., 2003), Alzheimer's disease (Fotiou et al., 2000), Depression (Fountoulakis et al., 1999), Down's syndrome (Sacks and Smith, 1989) and Generalized Anxiety Disorder (Bakes et al., 1990).

In general, the size and responsiveness of the human pupil are governed by the antagonistic action of the sphincter and the dilator muscles of the iris that are controlled by the Parasympathetic (ParNS) and the Sympathetic (SNS) Nervous System, respectively (Fotiou et al., 1998).

Alzheimer's disease (AD), the most common cause of dementia, is impossible to diagnose precisely without invasive techniques, particularly at the onset of the disease. Two of the earliest symptoms of AD are memory loss and decreased cognition. Some investigators estimate that fewer than half of all AD patients are currently diagnosed (Solomon and Murphy, 2005).

In regards to Parkinson's disease, studies have demonstrated that over 50% of PD patients have some mild intellectual impairment, even in the early stages of the disease. The patients are usually aware of the change in their cognitive functions and develop adaptive strategies to compensate for it (Green, 1999). As many as 15–30% of patients may even have severe symptoms or dementia. The cognitive impairment has usually, but not always, a prefrontal–subcortical pattern (Boller and Traykov, 1999). It includes the following deficits: a) disorders of executive functions, b) visuospatial difficulties, c) retrieval deficits and difficulty in recalling stored information, d) reduced ability in acquiring new information, e) abnormalities in procedural memory and f) difficulty in the use and comprehension of complex sentences (Pirizzolo et al., 1982; Cummings et al., 1988; Cummings and Huber, 1992; Dubois and Pillon, 1997).

The Pupillary Light Reflex (PLR) was first studied more than 40 years ago with pharmacological methods (Ascher, 1962, 1963) and was used to evaluate Adie's tonic pupil (Thompson, 1977). Following this, its use has broadened markedly to include patients suffering from AD (Idiaquez et al., 1994; Pomara and Sitaram, 1995; Scinto et al., 1994; Loupe et al., 1996; Treloar et al., 1996; Kardon, 1998; Marx et al., 1995; Sacks and Smith, 1989; Prettyman et al., 1997). The evidence concerning the pupil's reaction to light in PD is limited and controversial. Granholm et al. (2003) observed an increase of pupil's diameter in PD patients by using a tropicamide 0.01% solution. Pupil's diameter remained unchanged across time, especially in darkness. Beaumont et al. (1987), Harris (1991) and Micieli et al. (1991) found that PD patients developed reduced peak constriction amplitude (PCA), similar to AD patients (Beaumont et al., 1987; Micieli et al., 1991). However, whether PD patients had any psychiatric disorders or cognitive deficits is not mentioned in these studies, whereas, Fotiou et al. (2003) and Giza et al. (2006) studied pupillometry on nine and 27 PD patients respectively with no coexisting mood disorder or cognitive impairment.

The aim of the present study was to evaluate central cholinergic dysfunction, using fast video (263 frames/s) pupillometry, in patients with Alzheimer's and Parkinson's disease accompanied or not by cognitive impairment.

2. Subjects and methods

2.1. Participants

Twenty-three (10 men, 13 women), white, Caucasian normal control subjects (mean age 71.87 ± 8.51 years) and twenty-three (10 men, 13 women), white, Caucasian, probable AD patients (mean age 72.39 ± 5.41 years) entered the study. The 23 early diagnosed AD patients were selected from a group of 45 consecutive patients suffering from memory and cognitive disorders, who were followed up in the outpatient clinic of our department.

The criteria for selection were those of the NINCDS-ADRDA (NINCDS-ADRDA Work Group, 1984) and subjects assessed with the Mini-Mental State Examination (Altman, 1991). The probable AD patients underwent electroencephalogram, pattern reversal visual evoked potentials (PRVEP), electroretinogram (F-ERG), brain MRI and SPECT.

On the other hand, the Parkinson's disease group was formed by twenty two (22) patients (10 males, 12 females), aged 72.7 ± 7.3, from the outpatient clinic of our department. The PD patients, who entered the study, were white Caucasian with identified PD based on the results from the clinical examination, the UPDRS scale and the DAT-SCAN. They were examined with the Mini Mental State Examination (MMSE) and their Wechsler II scale score was calculated, in order to determine whether a cognitive impairment coexisted. According to the aforementioned scales, eleven (11) patients (PDNoCog group, five males, six females, mean age: 72.09 ± 7.06 years) were free of any cognitive deficits and eleven (11) patients (PDCog group, five males, six females, mean age: 73.36 ± 7.55 years) had cognitive disorder. The Hamilton Depression Scale ($HAM\text{-}D_{17}$) was applied and depression or anxiety disorder was not found in any of the 22 patients. The mean duration of their disease was 5.1 ± 4.3 years. Concerning their treatment, they received l-dopa and/or dopa agonists. They did not receive any anticholinergic, cholinomimetic or antidepressant agents.

There were two control groups. Both consisted of healthy volunteers, members of the hospital staff or their relatives, with no prior history of cognitive impairment. The first group (N1) consisted of 23 volunteers, matched for age and sex with the AD patients. In the second group (*N2*), used as control group for the PD patients, 22 volunteers participated and they were also matched for age and sex with the PD group.

All participants (control groups, probable AD patients and PD patients) provided their written informed consent, and all experiments were approved by the Ethics Committee of the AHEPA University Hospital according to the Helsinki Declaration. All were also free of any other neurological or ophthalmological disease, as determined before inclusion, and had symmetrical pupils. Participants underwent standard blood and biochemical laboratory tests. Those with past history of ocular operations or diseases affecting pupillary reflexes, and those receiving cardiac glycosides, anticholinergics, sympathomimetics, ß-blockers or other agents affecting heart rate variability or ophthalmic agents affecting PLRs were excluded. All patients underwent pupillometric examinations before treatment with cholinomimetic drugs.

2.2. System of pupillometry

The system for recording the pupil reaction to light is monocular, fully automated, and consists of the following items:

- a CCD high-speed digital camera connected to a computer (with up to 263 frames/s, with maximum responsitivity in the red and infra-red regions of the spectrum)
- a computer and associated sampling cards
- light sources and more precisely, two kinds of independent light sources; a) an infra-red light source, illuminating the face of the person, consisting of an array of 32 LED with maximum intensity at 820 nm and switched on throughout the measurement, b) a clinical photic stimulator (SLE; BioLogic, system corporation U.K). A flash is produced by a light bulb, through the discharge of a capacitor, of 20 ms duration. Diffuse light is shed in the whole visual field, which predominantly affects the posterior pole of the retina.

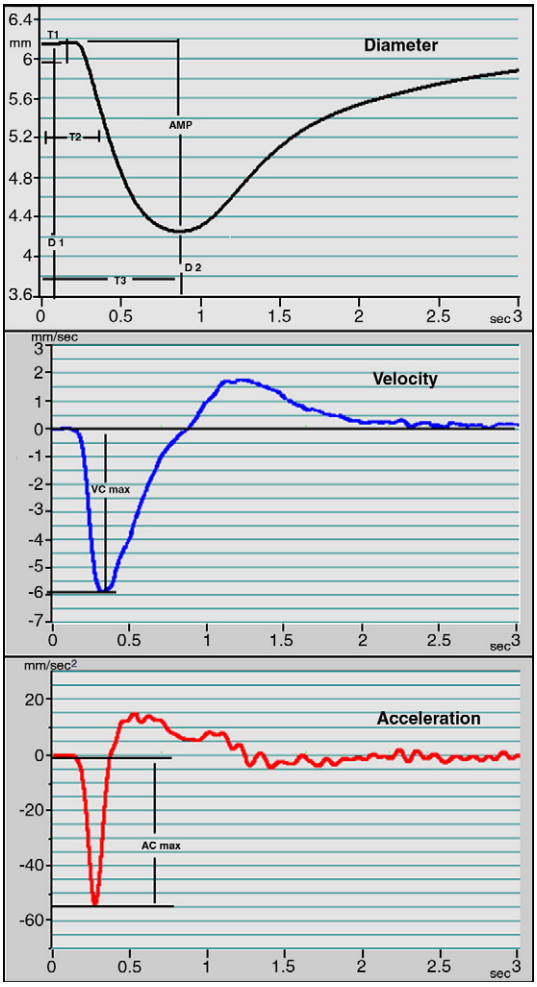

Fig. 1. Parameters measured — Baseline Pupil Diameter (*D*1), Latency (T1), Minimum Pupil Diameter (*D*2), Amplitude (AMP), Maximum Constriction Velocity (VCmax), Maximum Constriction Acceleration (ACmax), Time for Maximum Velocity (T2), Time for Maximum Constriction (T3).

- a traversing mechanism; the whole instrument is based on an optical examination table with a head-rest fixing the position in space of the head on one side. The camera is fixed on the top of the table, on a mechanism which can move in three directions, *x*–*y*–*z*. The camera can also rotate on both *x*–*y* and *x*–*z* planes
- image processing analysis software, which calculates pupil reflexes in real-time (up to 263 frames/s, with maximum responsitivity in the red and infra-red regions of the spectrum). Because of the corneal curvature, and in order to avoid errors due to optical distortion, the camera was set normal to the axis of the eye and at a distance of 30 cm, in order for the image of the pupil to be symmetrical.

2.3. Accuracy of measurements

The accuracy of measurements depends on the number of pixels covered by the pupil itself, which in turn depends on the optics of the system. In the present case, spatial resolution in both *x* and *y* directions was 0.015 mm. Temporal resolution was 2 ms.

2.4. Experimental conditions

The conditions in which the examination would take place were explained to each participant in detail. A first trial examination of the eye followed, in order to familiarize participants with the environmental conditions. Each eye was tested separately. Along with the control groups, both AD patients in a primary stage and PD patients, performed the experiment with proper cooperation, and the whole examination followed, with not many trials dropped by the examiner. Very rarely, the examiner was obliged to drop a couple of trials before saving a patient's examination. Subjects fixated an infra-red light in the same axis with the camera at a distance of 1.5 m. Participants remained for 2 min in complete darkness, after which 5 rectangular flashes of light at 30-s intervals were administered. A full record was completed in the 30-s interval and during this period, it was decided whether this recording should be rejected or saved (when free of any artefacts). Stimulus duration was 20 ms, and the luminance 24.6 cd/m2. The SLE flash (BioLogic, system corporation U.K) was placed in front of the eye under examination at a distance of 30 cm. A full record of the pupil's reaction diameter and centre, as a function of time, was made and then analysed on line. These data yielded the latency, velocity and acceleration, calculated as a function of time as well as other relevant parameters.

Calculated parameters were (Fig. 1):

- baseline pupil diameter after 2-min dark adaptation(*D*1);
- latency (onset of pupil reaction to light) (*T*1);
- minimum pupil diameter after pupil reaction to light (*D*2);
- amplitude (*D*1–*D*2) (AMP);
- VCmax;
- ACmax;
- time for maximum velocity (*T*2);
- time for maximum constriction (*T*3);
- percentage recovery–redilatation (*D*1%);
- percentage amplitude (%*D*1–*D*2) (%AMP).

The recording of the measurements was necessary in order to obtain the above parameters covering the changes in the size of the pupil for a period of 3.5 s from the application of the light flash. The *D*1% and %*D*1–*D*2 is the ratio of the initial resting pupil size to the final measurement after 3.5 s. Latency is defined as the time of maximum acceleration of the pupil, and the time of maximum myosis, is the time of zero (0) constriction velocity.

2.5. Statistical analysis

Due to the nature of the dependent variables (continuous), parametric methods were used for data analysis. Continuous data were first assessed for normality, using the Kolmogorov–Smirnov test. Since data were normally distributed, they were summarized with means and standard deviation (SD) or 95% confidence intervals (CI) for each PLR variable for both control groups, PD and AD patient groups. Categorical variables (sex) were compared with Cochran's *Q* test and age was compared with repeated ANOVA measures due to the matching of each control group with the corresponding patients' group. Measurements of 5 artefact-free (averaged) pupil light

Table 1
Classification accuracy of Pupil Light Reflex (PLR) parameters between normal and AD patients.

PLR parameters	AUC	95% CI	*p*-value*
ACmax	1.000	1.000–1.000	<0.001
VCmax	0.998	0.992–1.000	<0.001
AMP	0.955	0.886–1.000	<0.001
T1	0.914	0.832–0.996	<0.001
T3	0.907	0.798–1.000	<0.001
T2	0.896	0.803–0.989	<0.001
%AMP	0.888	0.796–0.981	<0.001
%*D*1	0.771	0.634–0.909	0.002
*D*1	0.696	0.544–0.847	0.023
*D*2	0.543	0.373–0.712	0.621

AUC: Area under the curve, CI: Confidence interval.
*$p<0.005$ considered significant (Bonferroni correction).

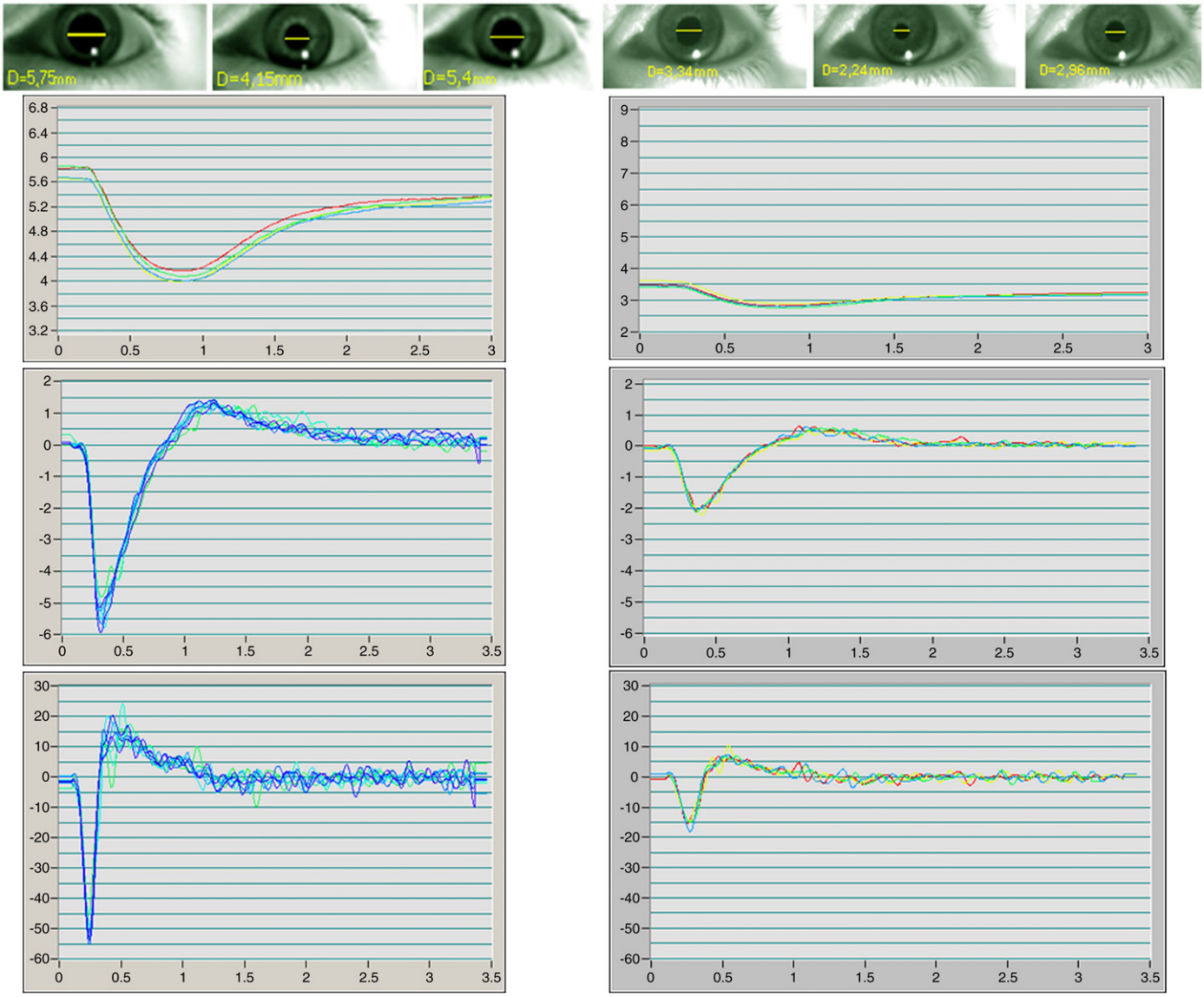

Fig. 2. The pupil size after a 2-min dark adaptation before the pupil's reaction to light, the maximum constriction and re-dilatation as a response to the light stimulus. We can see the difference in Baseline Pupil Diameter after 2-min dark adaptation (*D*1), the Maximum Constriction Velocity (VCmax), Maximum Constriction Acceleration (ACmax) in both normal subject and AD patient.

response curves of the right eye were used, as no statistical difference was found between the two eyes ($p>0.89$) (Sullivan Pepe, 2004).

Lastly, paired *t*-test analysis was used to examine differences between the mean scores of the matched groups (AD or PD group with the corresponding control group) for all dependent variables. In order to illustrate the classification accuracy of the PLRs, we also performed receiver-operating characteristic (ROC) curve analysis. The area under the curve (AUC) of the ROC curves was estimated and used as the index of classification accuracy, in which a variable with an AUC = 1 indicates perfect classification ability into one of the two diseases (AD or PD), or into the normal group. In the case of PD groups, the indexes of classification ability were also calculated for classifying a patient into the PD group with cognitive impairment (PDCog), or into the PD group with no cognitive impairment (PDnoCog). On the other hand, a

Table 2
Classification accuracy of the Pupil Light Reflex (PLR) parameters between PD patients without cognitive impairment (PDnoCog) and normal subjects.

PLR parameters	AUC	95% CI	*p*-value*
ACmax	1.000	1.000–1.000	<0.001
VCmax	0.992	0.965–1.000	<0.001
AMP	0.855	0.682–1.000	0.005
T1	0.789	0.583–0.995	0.022
T2	0.727	0.485–0.970	0.071
*D*1	0.591	0.343–0.839	0.470
*D*2	0.504	0.249–0.759	0.974

AUC: Area under the curve, CI: Confidence interval.
*$p<0.002$ considered significant (Bonferroni correction).

Table 3
Classification accuracy of the Pupil Light Reflex (PLR) parameters between PD patients with cognitive impairment (PDCog) and normal subjects.

PLR parameters	AUC	95% CI	*p*-value*
ACmax	1.000	1.000–1.000	<0.001
VCmax	1.000	1.000–1.000	<0.001
AMP	1.000	1.000–1.000	<0.001
T1	0.913	0.750–1.000	0.001
*D*2	0.835	0.648–1.000	0.008
T2	0.636	0.379–0.893	0.279
*D*1	0.579	0.330–0.827	0.533

AUC: Area under the curve, CI: Confidence interval.
$p<0.002$ considered significant (Bonferroni correction).

Table 4
Classification accuracy of the Pupil Light Reflex (PLR) parameters between PD patients without cognitive impairment (PDnoCog) and patients with cognitive impairment (PDCog).

PLR parameters	AUC	95% CI	*p*-value*
ACmax	0.967	0.896–1.000	<0.001
VCmax	0.963	0.892–1.000	<0.001
AMP	0.950	0.866–1.000	<0.001
*D*2	0.777	0.581–0.973	0.028
T1	0.715	0.489–0.941	0.088
*D*1	0.620	0.379–0.860	0.341
T2	0.579	0.328–0.829	0.533

AUC: Area under the curve, CI: Confidence interval.
$p<0.002$ considered significant (Bonferroni correction).

variable with an AUC near 0.5 indicates poor classification ability (Bergamin and Kardon, 2003). The Bonferroni correction was used to adjust for multiple comparisons, and reported p values<0.005 were considered as significant ($\alpha = 0.05/10 = 0.005$). Analyses were conducted in SPSS 14 (SPSS, Inc., Chicago, IL).

3. Results

Age and gender were well balanced between groups of AD and N1 and between PD group and *N*2. Paired *t*-test analysis was employed to examine the differences between the mean scores of each patient group and the corresponding control group for all PLR variables.

The analysis revealed significant differences between AD and N1 group for eight out of ten PLR variables ($p<0.001$ for all 8 comparisons). Specifically, AD patients had significantly lower levels of VCmax, ACmax, AMP and %AMP compared with normal subjects; conversely, AD patients had significantly higher levels of %*D*1, *T*1, *T*2 and *T*3 compared with normal controls. There were no significant differences between the two groups for *D*1 and *D*2 variables ($p=0.017$ and $p=0.423$, respectively).

Table 1 provides evidence of the classification power of each PLR variable in discriminating the participants of the two groups (AD and *N*1). ACmax was the best predictor in classifying a participant as normal or AD, with perfect classification ability (AUC = 1), leaving VCmax in second place, with almost perfect classification ability (AUC = 0.998), and AMP and *T*1 in third and fourth place, also with high classification ability (AUC = 0.955 and AUC = 0.914, respectively). *D*1 and *D*2 were ranked last with a lower AUC, indicating poor classification power in discriminating the two groups ($p=0.023$ and $p=0.621$, respectively). Fig. 2 shows the average curves of VCmax, ACmax and *D*1 of the right eye in normal subjects and AD patients after 2-min dark adaptation, before and after the pupil's reaction to light.

As for the *t*-test analysis on PD and *N*2 groups, ACmax and VCmax were significantly lower in patients without cognitive impairment compared to normal controls. The other PLR variables did not differ significantly between the two patient groups. Patients with cognitive deficits had even lower ACmax and VCmax levels compared with normal controls (*N*2) and additionally, AMP differed significantly between the two groups. Comparing the two PD groups, patients with

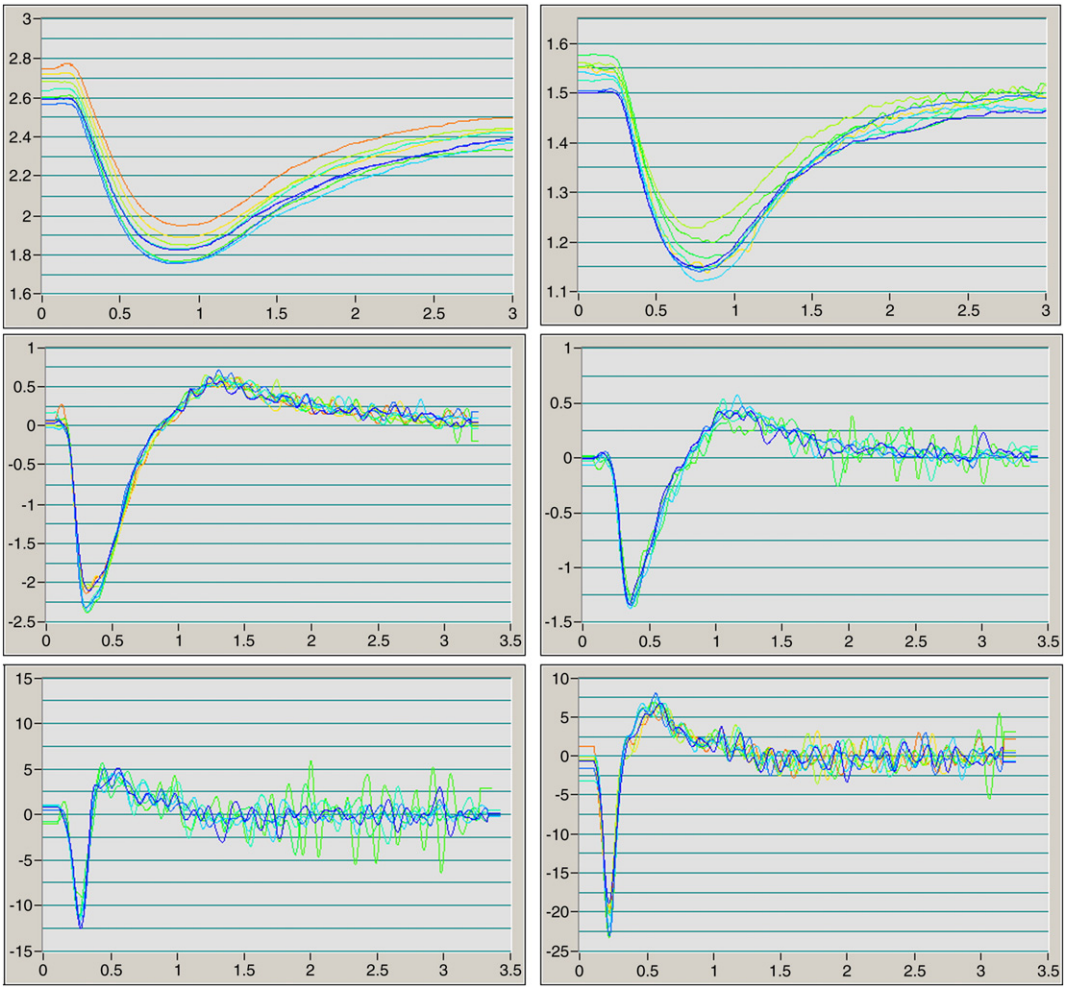

Fig. 3. Comparison of the pupillometric findings between a 65 year old patient with PD (UPDRS:35) and coexisting cognitive impairment (MMSE:22, Wechsler II:31,5) and a 67 year old healthy subject. The PD patient shows pathological findings in Amplitude (*D*1–*D*2), Maximum Velocity (Vcmax) and Maximum Acceleration (Acmax).

cognitive impairment had significantly lowers levels of ACmax, VCmax and AMP than patients without cognitive deficits ($p \leq 0.001$ for the 3 comparisons).

Tables 2–4, provide an evidence of the classification power of each PLR variable in discriminating the participants into one or the other group. ACmax was the best predictor in classifying a participant as normal or as a PD patient without cognitive impairment with a perfect classification ability (AUC = 1; Table 2), leaving in the second place, VCmax, with almost a perfect classification ability (AUC = 0.992). In classifying a participant as normal or as a PD patient with cognitive impairment, ACmax, VCmax and AMP were ranked first with a perfect classification ability (AUC = 1; Table 3), leaving in the second place T1 with almost a perfect classification ability (AUC = 0.913). Fig. 3 shows the average curves of *R*1–*R*2, Vcmax and Acmax in normal subjects and PDCog group.

Finally, ACmax was the best predictor in classifying a PD patient into one or the other group (PDCog or PDnoCog) with the highest classification ability (AUC = 0.967; Table 4). Furthermore, VCmax provided an almost perfect classification (AUC = 0.963) and in the third place, AMP appears as an additional classifier (AUC = 0.950).

4. Discussion

The pupil's constriction is governed by the parasympathetic branch of the Autonomic Nervous System (ANS). Therefore, the investigation of the changes in pupil size and mobility during the phase of constriction can be used as an accurate method in order to assess the function of the corresponding neurotransmitter, namely acetylcholine (Fotiou et al., 2007; Loewnfeld, 1999; Wilhelm and Wilhelm, 2003). Several studies have suggested that pupillometry is a sensitive technique for the investigation of early cholinergic deficits, which generally reflect an impending impairment in cognitive functioning. Additionally, studies in AD patients have implied that their pathological pupillometric findings can be attributed to a central cholinergic deficit. It has been proposed by researchers that pupillometry can be used as a diagnostic tool in the early stages of AD (Prettyman et al., 1997; Fotiou et al., 2000a,b, 2007; Scinto et al., 1994; Bitsios et al., 1996).

With reference to AD, the existing cholinergic deficit may give rise to the great differentiation of the results between the two groups (AD group and N1 group), especially the ACmax parameter. In our study, this parameter showed perfect classification ability in discriminating a participant as normal or AD and a clear-cut reduction in the AD group was recorded.

As described in the results, our AD patients had significantly lower levels of VCmax, ACmax, AMP and %AMP compared with normal controls; conversely, AD patients had significantly higher levels of %*D*1, *T*1, *T*2 and *T*3 compared with normal controls. There were no significant differences between the two groups for *D*1 and *D*2 variables. The low levels of VCmax may confirm the cholinergic deficit in AD, since ACh is the main neurotransmitter during myosis in PLR. In other words, the sluggish response of the PRL in AD may be due to a cholinergic deficiency.

The evidence concerning the PLR in PD is limited and controversial. As mentioned above, Granholm et al. (2003) found an increase in pupil's diameter by using a tropicamide 0.01% solution. Beaumont et al. (1987), Harris (1991) and Micieli et al. (1991) observed that PD patients developed reduced peak constriction amplitude (PCA). However, the aforementioned researchers used different means of investigating the pupil's reaction in their studies. Furthermore, it was not mentioned whether their patients had any coexisting psychiatric disorders or cognitive deficits. Previous studies (Fotiou et al., 2000a; Giza et al., 2006) indicate the presence of pathological pupillometric findings in PD patients, concerning VCmax and ACmax. The PD patients of those previous studies did not have any detectable cognitive deficits or psychiatric disorders.

In our study, the sample of 22 PD patients were divided in two groups according to the results of MMSE and Wechsler II scale: 11 patients without any cognitive deficits (PDnoCog) and 11 patients with coexisting mild to severe cognitive impairment (PDCog). None of the patients had depression or anxiety disorder according to the results of the HAM-D_{17} scale. We compared the pupillometric findings of each group with an age and sex matched group of 11 healthy subjects (*N*2) and an additional comparison between the findings of the two groups PDCog and PDnoCog was also conducted.

According to the procedure and the experimental conditions described above, VCmax and ACmax were found significantly lower in both groups of PD patients compared to the control group. These findings replicate those of Fotiou et al. (2000a) and Giza et al. (2006). The comparison of the pupillometric findings between the two groups of PD patients showed that the group with coexisting cognitive decline had significantly lower levels of AMP, and especially VCmax and ACmax, than the group without cognitive disorder. The findings of the group with coexisting cognitive deficits were almost similar to those of AD patients of previous studies (Prettyman et al., 1997; Fotiou et al., 2000a, 2007; Kalliolia et al., 2006).

It is important to note that primarily ACmax and secondarily VCmax were the best predictors in discriminating a participant as normal or as a PD patient (with or without cognitive impairment). According to Yamaji et al. (2000), the parameters involved in the first segment of the characteristic V-shaped pupillometric response, which is governed exclusively by the parasympathetic branch of the ANS, are VCmax and ACmax. These parameters can be considered as the most sensitive pupillometric markers of the cholinergic activity. Therefore, the lower levels of the above parameters may indirectly reflect an underlying cholinergic deficit.

The findings of prior pupillometric studies have been attributed to the following three possible pathophysiological mechanisms: a) the neuronal loss and gliosis of Edinger–Westphal nucleus, b) the increased inhibition from cortical, diencephalic and mesencephalic structures of the aforementioned nucleus and c) the functional impairment and degeneration of amacroinic, dopaminergic cells of the retina, which are important components of the PLR (Harris, 1991; Ikeda and Head, 1994; Witkovsky, 2004).

All of the AD and PD patients in our study were free of any ophthalmological disease, had corrected visual acuity of 10/10 in each eye, symmetrical pupils and normal F-ERG and PR-VEP. Therefore, we can assume that their pupillometric findings may not be attributed to the functional impairment or degeneration of amacroinic, dopaminergic cells of the retina. On the other hand, we can conclude that an additional (and perhaps the most significant) pathogenetic factor for these findings may be the involvement of a central cholinergic deficit, which leads to memory and cognitive impairment. This deficit can be detected by pupillometry, even perhaps in the early stages of the disease. The early detection of memory and cognitive impairment and thus of acetylcholine deficiency, promises to be of high importance in the therapeutic approach and prognosis of AD and PD.

5. Conclusion

Pupillometry is a low-cost, non-invasive technique, which exhibits a broad range of clinical applications. It is widely used as a clinical test for the assessment of the function of both branches of the ANS. Pupillometry is a sensitive technique for the investigation of early cholinergic deficits, which generally lead to memory and cognitive disorders.

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