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Repetitive Pupil Light Reflex: Potential Marker in Alzheimer’s Disease?

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Abstract. It was investigated whether alterations of the pupil’s light reflex might reflect Alzheimer’s disease (AD) pathology. Changes in the pupil’s system might be expected due to AD pathology present in the oculomotor system of the Edinger-Westphal nucleus, and a cholinergic deficit caused by degeneration of the nucleus basalis Meynert. A rather new method of repetitive light stimulation was applied assessing variations in pupil size, latency, and amplitude over time. We analyzed 44 healthy controls, 42 subjects with mild cognitive impairment (MCI), and 66 AD patients. AD and MCI showed a less pronounced pupil size decrease and amplitude increase over time than controls. A higher MMSE was associated with a higher increase of relative amplitude and greater decrease of latency in AD and MCI, and absolute amplitude increase in AD alone. Pupil size increase correlated with cerebrospinal fluid markers in AD. Summarized pupil light reflex is not stable under repetitive stimulation, but changes systematically and less pronounced in AD and MCI. Thus repetitive stimulation of the pupil’s response potentially indicates AD pathology.

Keywords: Alzheimer’s disease, amyloid-β 1-42, cerebrospinal fluid, mild cognitive impairment, parasympathetic system, pupil light reflex, repetitive stimulation, sympathetic inhibition, tau

INTRODUCTION

Alzheimer’s disease (AD) is a devastating disease characterized by progressive deterioration of more and more parts of the brain with initial memory loss and subsequent affection of virtually all cognitive functions as well as behavioral abnormalities. There is an estimated number of about 11 million people affected worldwide, and this number is assumed to almost double in the year 2025 [1]. Differentiating AD from normal aging can be difficult especially with regard to the early stages. Besides history and clinical examination, neuropsychological investigation, neuroimaging with PET or SPECT, as well as cerebrospinal fluid (CSF) biomarkers such as tau or amyloid-β 1-42 (Aβ1-42) may be helpful in establishing the diagnosis [2]. However to date there is no simple and accurate test for differential diagnosis early on.

A histopathological examination of eight early-stage AD patients revealed tangles and Aβ-containing plaques in the Edinger-Westphal (EW) nucleus, which represents the visceral portion of the oculomotor complex and in addition to its role in controlling pupillary constriction may mediate between the sympathetic and parasympathetic innervation of the iris musculature [3]. In AD, a cholinergic deficit is regarded as the most relevant neurotransmitter deficit although occurring according to a large postmortem study lately in the course of the disease [4]. More specifically, the only cholinergic deficit in AD is found in the cortically projecting basal forebrain system which does not directly influence the pupillary system and in the EW nucleus where a neuronal cell loss occurs [5]. Acetylcholine

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plays a major role in regulating the pupils. Indeed tropicamide which is a M4 receptor antagonist that competes with acetylcholine in binding to muscarinic receptors is usually administered to the eyes when ophthalmoscopy is performed in order to block cholinergic receptor sites and dilate the sphincter muscle of the pupil. Subjects with a normal level of acetylcholine do not react to very dilute tropicamide. On the other hand, in subjects with an acetylcholine deficit as in AD, the sensitivity of the acetylcholine receptors may be higher than normal because they are enabled to react to a smaller amount of acetylcholine. This assumption was supported by an investigation in 1994 that has gained a lot of interest. Scinto and colleagues found in patients diagnosed with probable AD a hypersensitivity to 0.01% dilute tropicamide and could differentiate patients from controls with a sensitivity of 95% and a specificity of 94% [6]. In the aftermath of the Scinto et al. study [6], several researchers attempted to confirm that AD patients displayed hypersensitivity to cholinergic antagonists and re-assessed the accuracy of the eye drop test.

Kardon [7] summarized the results of tropicamide eye drop tests from 19 previous reports [6, 8–25] including a total of 392 patients with AD and 498 controls. The author concluded that all but the initial study failed to demonstrate a significant difference of AD patients in the tropicamide eye test. In a reply by Scinto et al. [3], 29 published reports on tropicamide testing in AD were analyzed. In 16 [6, 8, 12, 13, 15–19, 21–23, 26–29] out of 24 studies comparing AD with healthy controls, pupil dilation was found to be more profound in AD than in controls [6, 8–19, 21–23, 26–33] but only in 10 of the reports did the findings reached significance [6, 8, 12, 15, 16, 19, 22–23, 26, 29]. As reasons for these conflicting results, Kardon named difficulties in the application and diffusion of the eye drop. In order to increase specificity, less concentrated tropicamide with 0.005% was recommended [34]. Others suggested the use of pilocarpin, a cholinergic agonist, instead of tropicamide which was reported to lead to an enhanced miotic response in AD patients [17, 35–38]. Furthermore three studies applied non-pharmacological tests to AD patients using either the pupil dark [39] or light reflex [39–41]. However, these studies suffered from small sample sizes and moreover delivered inconsistent findings. In sum, up to now the reports on pupil changes in AD can be called inconsistent at best.

A further source for the inconsistent findings is the variability of the pupil light reflex (PLR). Although PLR is frequently examined in neurology, psychiatry, ophthalmology, and other clinical fields, it is known to vary considerably; therefore more than a single stimulation is required in order to obtain a reliable result. However, it is not clear how far consecutive stimulation itself influences the PLR. Numerous confounding factors are described such as sensory stimuli [42], vigilance [42], respiration rate [43], emotional distress [42], day time [44], and age [45]. The latter is particular well examined and the pupil size typically decreases with age by 0.04 mm per year.

In this study the question was raised whether there are significant and systematic changes of PLR parameters during many (40) repeated measurements under well-controlled conditions in AD patients and individuals with mild cognitive impairment (MCI), a condition with an increased risk of later AD were compared to healthy controls. Apart from the repetitive measurement of the pupil light reflex, a further innovative aspect of the present study is that other biomarkers of AD, namely cerebrospinal fluid (CSF) tau and Aβ42, were taken into account. Finally, apolipoprotein E (APOE) genotyping and Mini-Mental Status Examination (MMSE) were included as covariates, as both measures relate to the risk and severity of AD, respectively [46].

**MATERIALS AND METHODS**

**Study population**

44 older healthy controls (mean age: 66.4 ± 8.3 years), 42 subjects with MCI (mean age: 69.8 ± 8.4 years) according to the Petersen criteria [47] and 66 patients (mean age: 74.4 ± 7.5 years) with AD (NINCDS-ADRDA criteria [48]) were included in this study.

By history and inspection, any ocular and neurological disorders and medications influencing pupillary function were excluded in our participants.

The study was conducted in accordance with the declaration of Helsinki.

**Pupil measurement**

In each of the subjects, 40 consecutive pupillary light reflexes of the left eye were recorded with a Compact Integrated Pupillograph (CIP; Fa. AMTech, Dossenheim, Germany). Measurements were performed under mesopic light conditions with a background luminance of approximately 1 cd/m². The CIP was focused on the iris of the left eye of each subject while the right eye fixated a small target in approximately 2 m distance. The CIP has an electronic “viewfinder” which allows con-
trolling focus and adjustment. A 585 nm light emitting diode placed above the front lens of the CIP served as a stimulus. Stimulus duration was 200 ms, brightness of the diode was 200 cd/m², and application was in a non Maxwellian-view manner. The subjects were allowed an adaptation time of 20 minutes.

The stimuli were applied with shortest interstimulus interval at least being 2 s. This interval was sometimes prolonged because of the necessity of focusing or adjusting with the pupillograph.

The following parameters were evaluated: pupil diameter, latency, amplitude, and relative amplitude (amplitude/pupil size). Pupil diameter is the pupil size before the start of the stimulus, the amplitude is the difference between initial and minimum diameter, and latency is the time from the stimulus onset till the beginning of pupil reaction. Different methods of analyzing the consecutive pupil response were piloted, for example the step of linear regression. We found that the comparison of the mean of the pupil response of the first and last five trials yielded the most consistent informative results. Therefore PLR parameters of the first and the last five measurements were averaged and compared.

As a composite score for cognition the MMSE [49] was entered into further analyses.

Cerebrospinal fluid analysis

CSF analysis was only performed in MCI and AD patients as part of their routine diagnostic work-up. CSF samples (6–8 ml) were obtained by lumbar puncture, collected in polypropylene tubes to avoid adsorbance of proteins to the test tube walls, and gently mixed to avoid gradient effects. The samples were centrifuged at 2000 g for 10 min to eliminate cells and other insoluble material, frozen and stored at −80°C pending biochemical analyses. No sample contained more than 500 erythrocytes/μL. CSF Aβ42 was determined using a sandwich ELISA (Innotest β-amyloid1-42; Innogenetics, Ghent, Belgium) constructed to specifically measure Aβ42 (Hulstaert). The microtubule-associated protein tau, a CSF marker of neuronal degeneration, was determined using a sandwich ELISA (Innotest hTau-Ag; Innogenetics) constructed to measure total tau, that is, all isoforms of tau irrespective of phosphorylation state [50], were measured.

APOE genotyping was performed by isoelectric focusing and subsequent precipitation by specific polyclonal antibodies and visualization by automated silver staining according to Hackler et al. [51].

Statistical analysis

Statistical analysis was performed using a software program (SPSS 21.0; SPSS Inc., Chicago, Ill). Multivariate analysis was performed to test for group differences in the basic demographic, pupil light reflex measures and CSF values. Dependent variables were pupil size, amplitude, latency, relative amplitude, CSF Aβ42 and tau; diagnosis was the independent variable and age the confounding variable. Differences of the parameters under repetitive stimulation were examined using an ANOVA with repeated measures with diagnosis as a between-subject factor and time (first and last measurements) as a within-subject factor. Pearson correlation coefficients were used to assess correlations among measures.

RESULTS

Basic characteristics

Repetitive stimulation of the PLR was applied to a sample of 44 older controls, 42 subjects with MCI and 66 patients with AD. Measured with the MMSE as a sum score for cognition, controls performed better than MCI (p = 0.001) and AD (p < 0.001), respectively. AD performed worse than MCI subjects (p < 0.001, Table 1).

In comparison of AD and MCI patients with healthy controls in Kruskal Wallis analysis, there were significant differences concerning age (χ² = 23.1, p < 0.001), initial pupil size (χ² = 21.5, p < 0.001), and amplitude (χ² = 6.2, p = 0.045), but not concerning latency (p > 0.2) or relative amplitude (p > 0.3).

Controls were younger than AD (p < 0.001) patients and there was a trend that controls were also younger than MCI subjects (p = 0.055). Moreover AD patients were older than MCI subjects (p = 0.12). To explore a possible impact of age on measures of the pupil light reflex Pearson correlation was applied. It was found that age correlated with most initial pupil measures (pupil size: r = −0.352, p < 0.001; amplitude: r = −0.205, p = 0.011; latency: r = 0.205, p = 0.011) but not with relative amplitude (p > 0.7) while age did not show an association to any changes of the pupil parameters under repetitive stimulation (p > 0.2). Therefore in further analysis of initial pupil light reflex measures age was included as a confound variable but not in the differences under repetitive stimulation.

In multivariate analysis using the initial pupil light reflex parameters as dependent variables in a significant model (F = 4.45, p = 0.002), only age remained as
Moreover there was a trend that in MCI pupil diameter (mean difference: 0.12, 95% CI: 0.26–0.01; p = 0.07) and significantly in AD (mean difference: 0.14 ± 0.06 mm, 95% CI: 0.26–0.01; p = 0.03). There were no differences between MCI and AD (p > 0.8).

Association of PLR and MMSE

Investigating any possible association with cognition as measured with the MMSE across all subjects an association was found for age (r = −0.339, p < 0.001), for initial pupil size (r = 0.261, p = 0.001), initial amplitude (r = 0.341, p < 0.0001), latency (r = −0.205, p = 0.012), and with a trend for relative amplitude (r = 0.148, p = 0.07). After correcting for age in partial correlation, a better cognition still remained associated with a larger pupil size (r = 0.151, p = 0.05), higher amplitude (r = 0.289, p < 0.001), and relative amplitude (r = 0.160, p = 0.05) and with a trend to a shorter latency (r = −0.140, p = 0.07). There was no association of the MMSE and the changes under repetitive stimulation (p > 0.5).

Analyzing the three diagnosis groups separately for controls, there was no association of cognition with age (p > 0.4), any change under repetitive stimulation (p > 0.1) or initial pupil light reflex parameters (p > 0.1) besides a trend for a higher amplitude (r = 0.273, p = 0.07). In MCI subjects, a better cognition correlated with a larger amplitude (r = 0.434, p = 0.005) and a higher increase of relative amplitude (r = 0.313, p = 0.05) and less pronounced decrease of latency (r = −0.287, p = 0.07) under repetitive stimulation. In AD patients, a higher MMSE was associated with a higher absolute amplitude (r = 0.358, p = 0.004) and relative amplitude (r = 0.351, p = 0.004), as well as with a more pronounced increase of amplitude (r = 0.303, p = 0.016) and relative amplitude (r = 0.365, p = 0.003), respectively, a more pronounced decrease of latency (r = −0.335, p = 0.007) under repetitive stimulation.

Association of PLR and CSF parameters

Finally in a subset of subjects, CSF analysis with estimation of tau and Aβ42 was available for analysis. Pupil data and CSF biomarkers were available in four healthy subjects in which diagnostic work up had not confirmed the initial suspect of cognitive impairment. Data of these subjects were compared to our patient samples (note that no lumbar puncture was taken).
performed in our main healthy control sample). In the four healthy subjects with available CSF data, tau was lower compared to the 40 AD patients (mean difference: 374 ± 131 pg/ml, 95% CI: 636–113, p = 0.006) while there was no difference to the 17 MCI subjects (p > 0.6). In AD, CSF tau was higher than in MCI subjects (mean difference: 304 ± 72 pg/ml, 95% CI: 448–159; p < 0.001) and MCI Aβ42 was lower in AD compared to controls (mean difference: 613 ± 130 pg/ml, 95% CI: 875–352 pg/ml, p < 0.001) and MCI (mean difference: 344 ± 71 pg/ml, 95% CI: 486–202; p < 0.001). In MCI subjects, Aβ42 was lower than in controls (mean difference: 269 ± 137 pg/ml, 95% CI: 544–6; p = 0.05). For all subjects, Pearson correlation found an association of a higher tau (r = −0.50, p = 0.02) and a lower Aβ42 (r = −0.458, p < 0.001) with a worse MMSE. There was no association of CSF markers to any of the initial pupil light reflex parameters (p > 0.1) and most of the changes under repetitive stimulation besides a relation of a lower Aβ42 to a less pronounced increase of relative amplitude (r = 0.244, p = 0.05). Since Aβ42 showed an age dependency (r = −0.26, p = 0.03) for the significant observations, a partial correlation was applied with age as confounding variable, where only an association of a better cognition with a higher Aβ42 remained (r = 0.391, p = 0.002). Analyzing MCI subjects and AD patients separately only a trend for a lower Aβ42 with a less pronounced increase of relative amplitude (r = 0.404, p = 0.09) was observed in MCI. Since there was an age dependency of tau (r = −0.560, p = 0.01), partial correlation was applied that revealed a trend for a higher tau to be associated with a less pronounced increase of pupil size (r = 0.444, p = 0.08). For all other PLR measures, no association with CSF tau was observed. In AD, a lower Aβ42 was associated with a smaller increase of pupil size (r = −0.381, p = 0.01). While there was no association of Aβ42 and age in AD, an older age was correlated to a lower tau (r = −0.355, p = 0.02). Therefore partial correlation was applied for CSF tau and PLR measures. The only association was found between a higher tau and a less pronounced increase of pupil size (r = −0.286, p = 0.08).

APOE did not have an association to initial PLR or change under repetitive stimulation.

**DISCUSSION**

Here we used the method of repetitive stimulation of the pupil light reflex to further shed light on conflicting results from former studies, which in some cases found differences in pupil reaction in AD patients compared to healthy controls, while in other cases no such difference was observed. We analyzed how these parameters change in pathological cases of MCI and AD and how they are related to CSF biomarkers of disease, cognitive abilities, and APOE4 genetics. However, as we applied the rather new method of repetitive stimulation, we will first discuss in detail the findings in healthy controls before we turn to the data of the patients.

**PLR in healthy aging**

During repetitive stimulation, healthy older controls showed a systematic change of pupillary light reflex parameters. Pupil size significantly decreased over time and latency became shorter; absolute and relative amplitude on the other hand increased.

Basically there are three possible explanations for this observation (Table 2). Firstly, it has been reported that after extensive stimulation, pupil size would not redilate to its initial size, the pupil would remain ‘captured’ (capture behavior; [52–54]). This would lead to a continuously smaller pupil size but not necessarily to a longer latency and altered contraction. In capture behavior, one would expect the amplitude rather to decrease and the latency to increase; however the opposite pattern was observed in the present study. A second explanation could be dark adaptation effects [55–56] due to insufficient adaptation time prior to the experiment. However, our examinations were performed in mesopic light conditions where only a short adaptation phase is required. Moreover, adaption to darkness should have resulted in an increase of pupil size where in reality we observed a decrease. Therefore adaptation effects can also be ruled out as an explanation for the observed changes. Hence, the third explanation is the most likely one: The systematic change of pupil light reflex over time is probably caused by decreasing sympathetic inhibition of the central parasympathetic system, an observation already published by Lowenstein and Loewenfeld in 1959 [57]. They observed that in a tired subject, pupil size decreased over repeti-
Fig. 1. Association of MMSE and changes of pupil light reflex parameters for MCI subjects (a-c) and AD patients (d-f). Amplitude increase showed only a significant association with a better cognition in AD patients (d) but not in MCI (a). There was a significant correlation of a higher relative amplitude increase and higher MMSE (b and e), and of a more pronounced latency decrease and a higher MMSE (c and f) in both groups.

Fig. 2. Association of pupil size increase and CSF parameters in AD. There was a significant association of pupil size increase and a higher Aβ42 (a) and a trend of an association to a lower CSF tau (b).

tive stimulation while latency seemed to get shorter and amplitude larger. Unfortunately no detailed values were provided in that work but only a copy of the original recording. More evidence in this regard comes from pharmacological studies where sympathomimetics are found to increase pupil size and to decrease amplitudes while parasympathomimetic acting drugs cause the opposite effects [58, 59]. In tired subjects under stable dark light conditions, pupil oscillations with consecutive decrease of pupil size occur not earlier than two minutes after start of the measurement and are strongly related to manifest sleepiness, e.g., emerge as a consequence of sleep deprivation [60]. Therefore although the applied design of repetitive pupil light reflex measurement can be assumed to be rather boring to the subjects, it is more a tiredness of the (para-)sympathetic system than of the subjects themselves that caused the observed effects as they occurred earlier than in the study by Wilhelm et al. [60].

Furthermore our data add information to the question whether or not age is a confounding factor regarding pupil light reflex parameters. For the pupil
diameter, an age-dependency is well documented in a large series of healthy subjects with a decrease of about 0.4 mm per decade [45]. For latency the available data are much less conclusive. Almost as many studies have demonstrated an age-dependency [59, 61–65] as have not [66–69]. Regarding the amplitude’s age dependency no clear evidence exists either. Our data suggest that there is an age-dependency for all parameters with a decreasing amplitude and prolonged latency with increasing age.

In sum pupil light reflex parameters like pupil size, latency, and amplitude are highly age-dependent in healthy subjects. Pupil light reflex is not stable over repetitive stimulation but underlies a systematic change that is most probable related to a changed interaction of the sympathetic and parasympathetic system (Table 2). These changes seem to be age-independent. Therefore repetitive pupil light reflex stimulation might not only serve as a model for studies of the (para-) sympathetic system, e.g., pharmacological studies, but seems to be preferable in investigations where an age-dependency should be ruled out.

PLR in MCI and AD

Having established how and why PLR changes with repetitive stimulation in healthy aging, we will now turn to the effects of neurodegenerative diseases on these parameters. Our MCI and AD patients showed no differences of initial PLR parameters compared to controls when corrected for age. Our finding is in accordance with previous observations of an unaltered pupil diameter in AD [39–41]. However, in other studies [39–41], a smaller amplitude was described in AD. The reason for the discrepant findings is presumably differences in measurement conditions and sample sizes. With our method of repetitive stimulation now a clearer picture emerges: Pupil changes over time were less pronounced in AD and MCI subjects compared to controls. Moreover, whereas MCI patients showed a correlation of MMSE with absolute amplitude, relative amplitude, and latency, a correlation of MMSE with absolute amplitude, relative amplitude, and latency, respectively, was not observed in AD.

In summary from our data some evidence derives that repetitive stimulation of the pupil light reflex is superior to initial measurement because it is age independent. Moreover it showed some association to AD pathology indirectly assessed with the MMSE and CSF Aβ and tau. As measuring the PLR repetitively is a rather simple and cost-effective assessment, it has the potential to aid earlier diagnosis of AD in the future.

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REFERENCES


