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Age-dependent pupillary light reflex parameters in children*

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Abstract— **Pupillary light reflex (PLR) refers to the phenomenon where pupil size changes in response to stimulation with a flash of light. It is a simple functional test that can reveal dysfunctions associated with the PLR pathway. Although abnormal PLR responses have been reported in many neurological disorders, few studies investigated neurodevelopmental effects on PLR parameters. We studied the effect of age on PLR in a group of 6 to 17 year old children with typical development. A significant and consistent age effect was found on PLR latency in children younger than 10 years old. Age effects were also observed in resting pupil diameter and constriction amplitude. However such age related trends were not observed in children with neurodevelopment disorders. These results suggest that PLR has the potential to be used as a simple noninvasive tool for monitoring neurodevelopment in children.**

I. Introduction

American Academy of Pediatrics (AAP) estimates that 12% to 16% children have some forms of developmental disorders [1]. Substantial clinical evidence supports that early intervention leads to improved functioning. Early detection is essential to ensure early intervention [1]. In the United States developmental screening is presumed be done in the pediatrician's or family doctor's office using one or more screening questionnaires [2]. Unfortunately, this practice is neither consistent nor universal which leads to considerable lag in the diagnosis for children with developmental disabilities [1]. In addition, behavioral symptoms usually lag behind the underlying neurophysiological changes. Therefore there is a need for an objective measure that can accurately track normal neurodevelopment progress in children.

Pupillary light reflex (PLR) is tested by measuring pupil size change in response to a short light flash. The size of the pupil is controlled by two antagonistic iris muscles: the sphincter and the dilator that are innervated by different neurological systems [3]. Photoreceptors in the retina detect and convey the sensory information about retinal illumination to the pretectal olivary nucleus (PON) via optic nerves. The PON synapses at the Edinger Westphal (EW) nucleus [4] which then projects to the ciliary ganglion to control the sphincter muscle via the short ciliary nerve [5, 6]. The neurological pathway related to pupil dilation is still not well understood [5]. The dilator muscle receives control from the superior cervical ganglion via the ciliary nerves. The ciliospinal center of Budge is found to project to the superior cervical ganglion [7].

PLR responses can be altered by dysfunctions in the PLR pathway. In fact, abnormal PLRs have been previously reported in several types of neurological disorders. Fan el al. [8] reported prolonged PLR latency, smaller relative constriction and lower constriction velocity related to autism spectrum disorder (ASD). Giza el al. [9] reported prolonged latency, reduced amplitude, maximum constriction velocity and maximum acceleration associated with Parkinson's disease. Fotiou et al. [10] reported atypical PLR associated with Alzheimer's disease, where all parameters except baseline and minimum pupil diameters were affected.

To develop an effective screen for neurodevelopment disorders, it is important to first understand neurodevelopment in typically developing children. Several studies have been conducted to examine the normal neurodevelopmental progress of the visual system in children by using visual evoked potentials (VEP) [11, 12]. A recent report demonstrated the potential of using PLR to examine visual system development in preterm babies [13]. However, no comprehensive study has been conducted to investigate age related profiles of PLR parameters in children.

Here we report our results of PLR tests in over 100 typically developing children from 6 to 17 years old. Our results revealed a significant age effect in PLR parameters, particularly the PLR latency and resting pupil diameter. A similar trend was not observed in a group of age-match children with neurodevelopmental disorders.

II. Procedure

A. Instrumentation

A custom-built binocular pupilogram recording system (Fig. 1) was used to measure PLR with high spatial (35μm/pixel) and temporal resolution (8.7 ms). The two recording channels are independent but synchronized. The optical stimulation and image acquisition were controlled through a computer interface via a custom-developed Labview program. This customized system has two "sighting" ports so that the participant can fix sight at a given target during PLR test. In addition, this system is versatile for

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setting various stimulation waveforms and intensities.

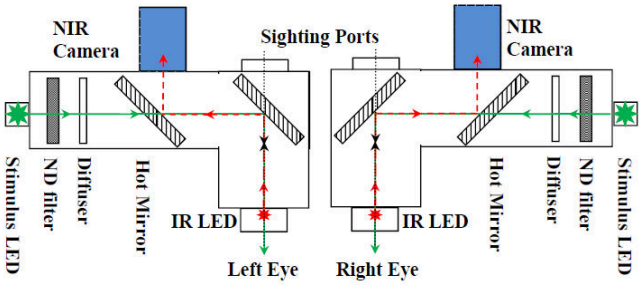

Figure 1. A schematic diagram of the binocular pupilogram recording system. A hot mirror was used in each channel to separate the optical stimulation path and imaging path. The participant can fix the sight on a monitor through the two sighting ports.

Pupils were illuminated by near infrared (NIR) LEDs at 880 nm wavelength. A 530 nm green LED was used to provide the light stimulus for evoking the PLR. The electric current to the LED was controlled to vary the stimulation irradiance along with the use of neutral density (ND) filters. The stimulation light then passed through a diffuser providing on-axis illumination with 5.7° visual field. The stimulation intensities used in this study varied from 0.09 $\mu W/cm^2$ to 9.9 $\mu W/cm^2$ in light-adaptation (LA) and was 0.09 $\mu W/cm^2$ in dark-adaptation (DA).

Two near infrared (NIR) cameras (GC660, Allied Vision Technologies, Stadtroda, Germany) were used in the system to acquire pupil images. The image size was 659 pixels × 494 pixels with a 12 bit resolution. At each PLR test, the cameras were triggered first to acquire baseline pupil images for 1s. Then the green LEDs were triggered to give a 100ms flash. Image acquisition was continued for four more seconds to capture the entire pupil constriction and recovery process. A total of 575 images were acquired from each eye in a single test trial (5 sec). All acquired images were saved using the tiff format.

Custom image processing software developed in visual c++ was used to automatically calculate the pupil diameter from each of the recorded pupil images in the image sequence (575 images for each eye). A histogram-based threshold method was applied after contrast stretching the pupil image to locate boundary pixels for the pupil. The threshold of pupil boundary was identified as the pixel value corresponding to first minima of the image histogram as shown in Fig. 2(b). Using this threshold the images were binarized and pupil was segmented. All pixels on the pupil boundary were then extracted. An ellipse was fitted to the segmented pupil boundary (Fig. 2(a)) by using a direct least square fitting algorithm [14]. The area of the fitted ellipse was used to estimate the pupil area. A nominal diameter was calculated by treating the pupil as a circle.

Once all pupil diameters were extracted from the acquired image sequence, a pupilogram curve (Fig. 3) was constructed to represent the pupil size change in response to the optical stimulus. The pupilogram was normalized against the resting pupil area to remove effects of resting pupil size when calculating constriction amplitude. The following PLR parameters were calculated from the pupilogram in Fig. 3 to quantify the pupillary response. The resting pupil diameter D_0 was calculated by averaging pupil diameters obtained during the 1s period before stimulus onset.

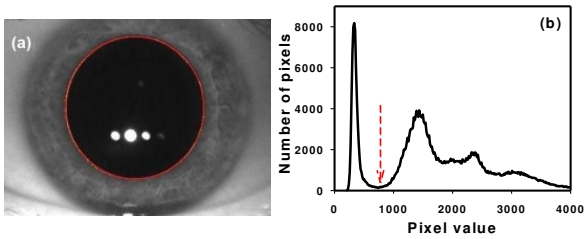

Figure 2. An example to illustrate the pupil segmentation used in our study. (a) An example pupil image. (b)The corresponding histogram. The first minima marked by the arrow in (b) indicates the boundary of the black pupil in (a). This value was used as the threshold to segment the pupil. The red circle in (a) shows the fitted ellipse using least square fitting.

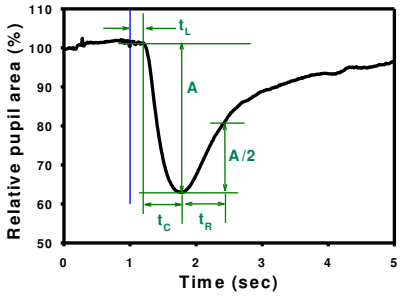

Figure 3. An illustration of the pupilogram which is normalized against the pupil area before stimulus onset (the resting pupil area D_0^2). Following extracted PLR parameters are shown: A= relative constriction amplitude; t_L = latency; t_C = constriction time; t_R= redilation time

The relative constriction amplitude was calculated by normalizing the difference between resting pupil area and minimum pupil area against the resting pupil area. PLR latency (t_L) was calculated as the time interval between stimulus onset and the beginning of pupil constriction. The constriction time (t_C) was calculated as the time interval between the beginning of pupil constriction and when pupil reached minimal size. The redilation time (t_R) was calculated as the time interval between the minimal pupil diameter and when the pupil recovered to half of the constriction. The pupilogram (before normalization) was smoothed by using a 6^{th} order Savitzky-Golay filter. To measure the PLR latency, the acceleration (2^{nd} order derivative) of the pupilogram was calculated. The time of the maximal acceleration was first identified and used as the starting point to back-track toward the stimulation onset. The first image frame that deviated from the baseline pupil size was considered as the onset of pupil constriction.

B. Test procedure

PLR data were obtained in 107 healthy children 6 to 17 years old (mean age 10.9±2.9 years) without any known visual or neurological problems. There were 79 males (mean age 10.9±3.1 years) and 28 females (mean age 10.6±2.4 years). As a comparison, PLR data were also examined in 176 children (mean age 10.5±3.1 years, 150 males and 26 females) with several different types of neurodevelopmental disorders including autism (147), mental retardation or developmental delays (10), Down's syndrome (7), Fragile

X syndrome (5), cognitive disorders (4) , learning disability (1), Prader Willi (1), Oppositional defiant disorder (1). This group of participants was recruited through the Thompson Center for Autism and Neurodevelopmental Disorders at University of Missouri. Written consents were obtained from all participants and their legal guardians as approved by the Institutional Review Board of University of Missouri-Columbia.

PLR was measured in both light adapted (LA) (room luminance of 30cd/m^2) and dark adapted (DA) (<0.02cd/m^2 room luminance) conditions. The intensities used as optical stimulation for PLR were 0.09μW/cm^2 in dark-adaptation, and 0.09μW/cm^2, 1.0μW/cm^2, 9.9μW/cm^2 in light adaptation. For each stimulus condition, PLR responses from both eyes were measured when one eye was stimulated. The measurements were repeated four times for each condition with an approximately 30s interval between two consecutive measurements. Imaging was started 1s before the stimulation to gather the resting pupil size. After the LA test, all participants stayed in the dark room for 15 minutes for the pupils to naturally dilate before starting the DA test.

C. *Data analysis*

The Analysis of Covaraince (ANCOVA) was applied in SAS to examine the effects of age and test conditions on each PLR parameter. Follow up analysis of variance (ANOVA) was performed to verify the age effect for a linear relationship. PLR parameters were verified for normal distribution using the Kolmogorov-Smirnov test. p<0.05 was considered as significant.

III. Results

As expected, in typically developing children the resting pupil diameter was larger in dark adaptation (7.44±0.77 mm) than in light adaptation (6.58±0.61 mm) as shown in Fig. 4. The resting pupil diameter increased with age significantly before 12 years old (F(6,135) = 2.67, p=0.018). From 6 to 12 years old, the mean resting pupil diameter increased 8.0% in LA and 13.2% in DA. The ANOVA test for a linear trend further confirmed that the age effect was significant (p=0.047 at LA and p= 0.003 at DA). At the same stimulus intensity, the PLR constriction amplitude was larger in dark-adaption whereas the constriction/redilation times were longer and latency was shorter. In light-adapted tests, as stimulus intensity increased from 0.09μW/cm^2 to 9.9μW/cm^2, PLR latencies decreased 21.85%; constriction and redilation times increased 25.30% and 48.15% respectively; and relative constriction amplitude increased from 11.76±5.54% to 40.75±7.23%.

The ANCOVA model suggested a significant age effect on several PLR parameters. In children from 6 to 8 years old, the age effect was significant for constriction amplitude (F(3,132)=3.48, p=0.018). PLR constriction increased with age in children younger than 8 years old and reached a plateau thereafter (Fig. 5a) at all stimulation conditions except the one at LA 0.09μW/cm^2. However the linear increasing trend at young age (< 8 years) was significant only with the maximal stimulus at LA 9.9μW/cm2 (F(1,21)=5.70, p=0.027). The PLR constriction time and the redilation time did not show an effect with age.

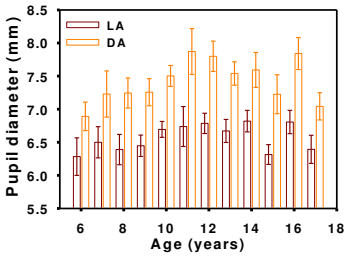

Figure 4. The age effect in resting pupil diameter in the light adapted (LA), and dark adapted (DA) environment in children with typical development. The error bars indicate the standard error.

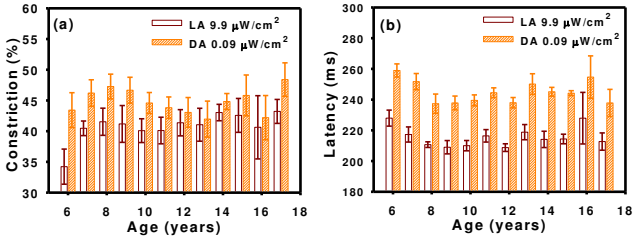

Figure 5. PLR parameters obtained in children with typical development from 6 to 17 years old. (a) Relative constriction amplitude, (b) latency measured in the light adapted (LA) 9.9μW/cm^2 and dark adapted (DA) 0.09μW/cm^2 condition. The error bars indicate the standard error.

The most consistent age effect was observed in PLR latency. The ANCOVA model revealed that in children from 6 to 9 years old, latency has a significant age effect (F(3,132) = 6.68, p<0.001). As shown in Fig. 5b, PLR latency decreased significantly at all testing conditions from 6 to 9 years and stabilized thereafter. For example, the PLR latency decreased from 259.0±4.3ms at 6 years old to 237.3±6.3ms at 8 years old at stimulation condition of LA 9.9μW/cm^2. The ANOVA test for a linear trend in children younger than 10 years old further confirmed that the age effect was significant (p< 0.01) at all conditions except the one with the lowest stimulus intensity of LA 0.09μW/cm^2.

Since we saw a consistently significant age trend in PLR latency, we examined the age trend in PLR latency and resting pupil diameter measured in a group of children of the same age range with neurodevelopment disorders. As shown in Fig. 6, no age dependent trend in PLR latency existed in this group of children. At the same stimulation condition, children with neurodevelopment disorders had significantly longer latency than typically developing children. Similarly we didn't observe any age effects on resting pupil diameter in this group of children.

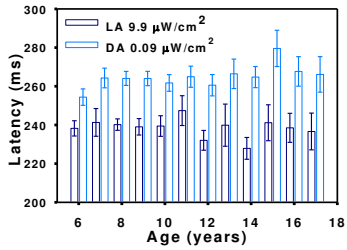

Figure 6. The PLR latency in children with neurodevelopment disorders measured at light adapted (LA) 9.9 μW/cm^2 and dark adapted (DA) 0.09μW/cm^2 condition. The error bars indicate the standard error.

IV. Discussion

PLR is an involuntary neurological response. Testing of PLR is noninvasive, simple and fast. It requires minimal cooperation from the subject and thus is convenient for testing in children. A good understanding of age dependent behavior of PLR is essential to evaluate the potential use of PLR for screening neurodevelopmental disorders in children.

Our results indicated a consistent and statistically significant age effect in PLR latency measured in young children (<10 years old) with typical development. These results appear to be consistent with previous findings of age-dependent changes in visual evoked potential (VEP) in children. Lenassi el al. [12] compared flash VEP and pattern VEP in infants and young children from 1.5 months to 7.5 years of age. They found that VEP latency for all three stimulation types showed an exponential decrease with age, but the trends were different. The latencies of reversal and pattern onset VEP showed fast decays (exponential decay rate of -9.3/year and -13/year respectively) and were stabilized by 6 months of age. However, flash VEP latency showed a slower decay (exponential decay rate of -0.54/year) and still decreased gradually at the upper limit of the age (7.5 years) they tested. Our age-dependent PLR latency in children (6 – 9 years) with typical development had a similar effect as the flash VEP latency results reported by Lenassi el al. [12]. Carrillo-De-La-Pena el al. [11] studied flash VEP in 85 children from 8 -15 years old and reported no significant age effect in latency. This result is consistent with our observation that PLR latency didn't change in children older than 9 years old.

Although a significant age effect was reported in relative constriction, it was statistically significant only at one test condition with the highest stimulus intensity. With a close examination, we noticed that the coefficient of variance for relative constrictions varied from 12% to 63% at those stimulation conditions where the age effect was not statistically significant. At the strongest stimulus of LA at 9.9 $\mu W/cm^2$, the coefficient of variance was much smaller, from 8% to 24%. Hence it is possible that the lack of statistical significance can be attributed to the higher variation in data obtained with smaller stimulus intensities.

The fact that no age-dependent trend in PLR latency or resting pupil diameter was observed in the group of children with neurodevelopment disorders suggests that the typical neurodevelopmental trajectory might be altered in neurodevelopmental disorders. The underlying mechanisms need further study. However, our result suggests that PLR has the potential to provide clinically useful information about progression of neural development in children.

V. Conclusion

We found a significant and consistent age dependent effect in PLR latency in children 6 to 9 years old. We also observed age effects in resting pupil diameter and PLR constriction amplitude. Such an age-dependent effect was not observed in children with neurodevelopment disorders. Further studies in larger groups of children especially in children younger than 6 years old are necessary to fully understand the details of age dependency of PLR. Nevertheless, PLR shows potential to be applied as a simple noninvasive tool to monitor neurodevelopment in children.

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