




# Pupillometry measures of autonomic nervous system regulation with advancing age in a healthy pediatric cohort

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## Abstract

**Purpose** To determine if variables of the pupillary light response mature with age and sex in a healthy pediatric cohort and the utility of pupillometry in assessment among pediatric participants.

**Methods** After 1 min in a dark room to establish baseline, pupillometry was performed on 323 healthy, pediatric participants (646 eyes; 2–21 years; 175 females). Variables included initial pupil diameter, pupil diameter after light stimulus, percent pupillary constriction, latency to onset of constriction, average constriction velocity, maximum constriction velocity, average dilation velocity, and time from light stimulus to 75% of the initial pupil diameter. Data analyses employed ANOVAs and non-linear regressions.

**Results** Analyses of age group differences revealed that participants 12–21 years old had a larger initial pupil diameter and pupil diameter after light stimulus, with males aged 12–18 years demonstrating a larger pupil diameter than all younger participants ( $p < 0.05$ ). Participants 12–18 years old had a slower maximum constriction velocity than participants 6–11 years old, with no sex differences ( $p < 0.05$ ). Furthermore, males aged 12–18 years old had a smaller percent constriction than males 6–11 years old ( $p < 0.05$ ). Regressions revealed that percent constriction and dilation velocity seemed to mature linearly, initial pupil diameter and ending pupil diameter matured quadratically, and the constriction velocity terms matured cubically.

**Conclusions** Results revealed maturation of the pupillary light response by age and sex in healthy pediatric participants. Given the value of the pupillary light response as a biomarker, the results provide normative benchmarks for comparison in health and disease, including opiate-exposed and concussion patients.

**Keywords** Biomarkers · Pupillometry · Pediatrics · Autonomic nervous system · Development

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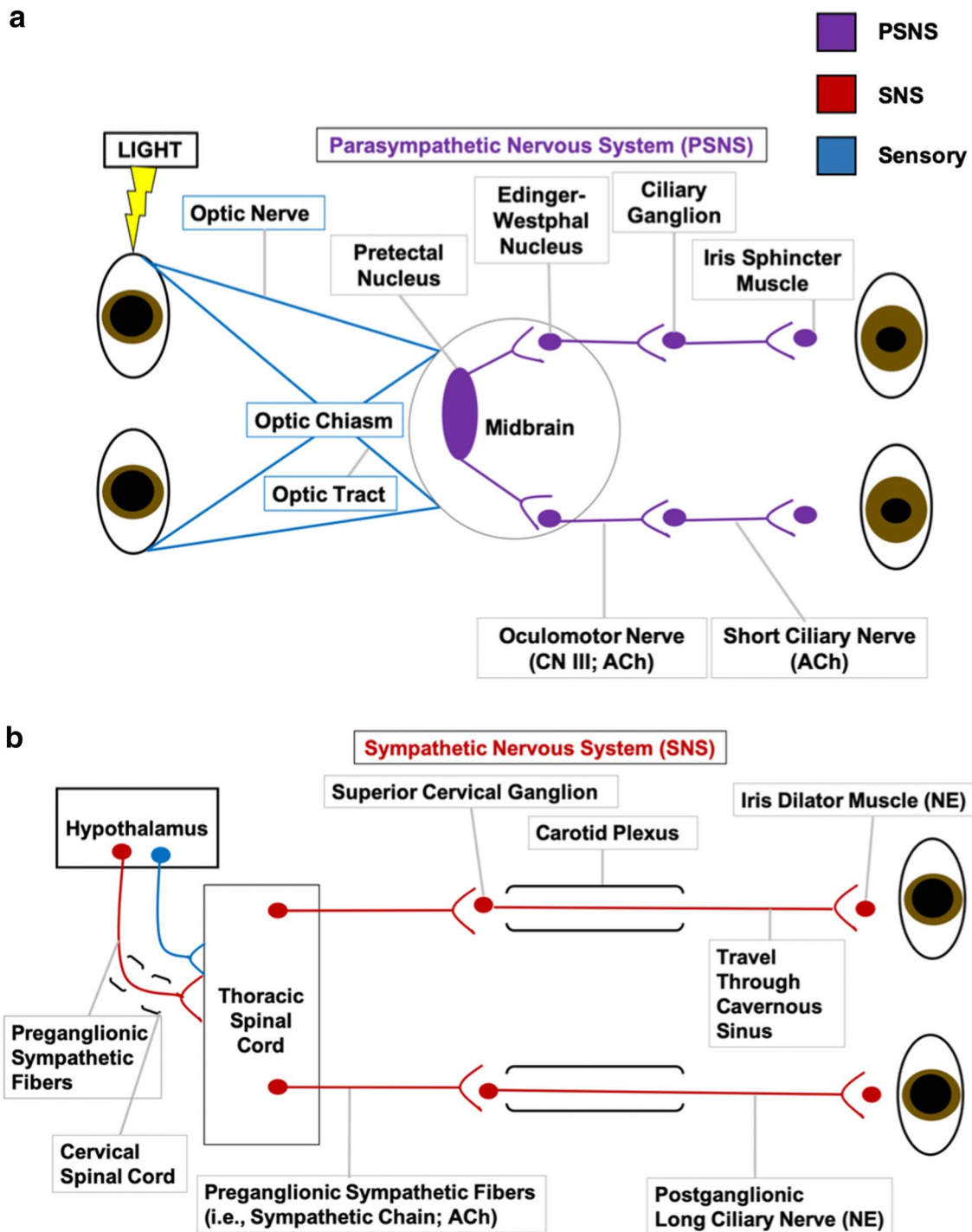
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## Introduction

The pupillary light response (PLR) is wholly automatic and cannot be consciously controlled, thereby offering the clinician and researcher an easily accessible non-invasive

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way to obtain objective autonomic nervous system (ANS) measures of parasympathetic nervous system (PSNS) and sympathetic nervous system (SNS) tone [1–5] (Fig. 1). Specifically, the PSNS component of the PLR begins after the pupil is exposed to light, with input traveling from the retinal ganglion cells and their axons to the pretectal nucleus and then, via the Edinger-Westphal nucleus and the third cranial nerve, terminating on the iris sphincter muscle, which

enables pupillary constriction [6–8]. The SNS contributes to the PLR after removal of the light stimulus with central sympathetic modulation of the Edinger-Westphal nucleus and peripheral sympathetic input to the iris dilator muscle, which originates in the hypothalamus and terminates at the pupil dilator muscle, resulting in active pupillary dilation (as opposed to just inhibition of constriction) [6, 8]. Specific neurotransmitters for the PSNS and SNS pathways are

**Fig. 1** Schematic of the pupillary light response (PLR). **a** After light is presented, the sensory information (blue) travels through the optic nerve to the pretectal nucleus in the midbrain, which then communicates with both Edinger-Westphal nuclei and begins the parasympathetic nervous system response (PSNS; purple). Efferent information then travels through the oculomotor nerve (CN III) to the ciliary ganglion and then to the iris via the short ciliary nerve and triggers constriction of the iris sphincter muscle and pupil. After the light stimulus has abated, central sympathetic modulation of Edinger-Westphal nuclei and peripheral sympathetic input to the iris dilator, which originates in the hypothalamus, begin the sympathetic response (red) and carry information to the thoracic spinal cord and preganglionic fibers. These fibers then synapse on the superior cervical ganglion, which allows motor information to travel to the iris dilator muscle and facilitates pupil dilation to accommodate dark adaptation. **b** This schematic illustrates the sympathetic nervous system (SNS; red) pupil dilation system, which occurs in dark acclimation. Sensory information (blue) is first carried from the hypothalamus to the thoracic spinal cord via preganglionic sympathetic fibers that sense dark vs. light. The signal then travels through the cervical spinal cord as preganglionic sympathetic fibers to the superior cervical ganglion. The signal then travels through the carotid artery plexus and cavernous sinus via the postganglionic long ciliary nerve to the iris dilator muscle where it facilitates pupil dilation. Neurotransmitters are provided in parentheses where appropriate: *ACh* acetylcholine, *NE* norepinephrine

acetylcholine and norepinephrine, respectively [9]. Moreover, focusing on nearby objects when convergence and accommodation are tested will activate the PSNS and cause pupil constriction. Prescription as well as non-prescription drugs and cognitive load [3, 10–13] will also impact the PLR. Considering the growing knowledge and utility of the PLR as a biomarker of autonomic regulation, and the introduction of an American Medical Association billing code in 2014 allowing mainstream clinical use of computerized pupillometry (CPT-0341T) in health and disease, establishment of normative values across the human lifespan is imperative.

Though hypothesized that components of the PLR mature throughout life, the maturational trajectory in a large pediatric cohort is under-described [5, 14]. Research in pediatrics suggests a slight increase in pupil size with advancing age [14], whereas research in older adults demonstrates a decrease in maximum pupil size and constriction velocity [7, 9, 15, 16]. While it is thought that this change may reflect a decrease in sympathetic tone [7], it can also be thought of as a change in the dynamic balance between the PSNS and SNS [17]. Despite the paucity of research on the maturation of the PLR, there is a growing body of literature that utilizes the PLR as a biomarker of a variety of neurologic, endocrine, and psychiatric conditions [9, 18–24]. Furthermore, the PLR is currently being explored as an indicator of intracranial pressure, concussion, and brain injury severity [25–29]. These studies are limited by a lack of consistency among their methods, control cohort size, and data analytics, collectively hindering their ability to draw generalizable

conclusions and contribute to reproducibility in research assessing the PLR and ANS (dys)regulation.

The present study aims to establish normative values in a pediatric cohort for the measurements that dynamically represent PSNS and SNS tone during the course of the PLR [24]. It tests the hypothesis that components of the PLR will mature with advancing age in healthy individuals aged 2 to 21 years old. Clinicians and researchers interested in utilizing pupillometry in their practices and research will be able to refer to these normative values for PLR sub-components to acquire a better understanding regarding how their patients and clinical cohorts, including opiate-exposed and concussion victims, differ from a healthy pediatric cohort.

## Methods

### Study participants

Healthy study participants were recruited and tested by the Autonomic Medicine research team at Ann & Robert H. Lurie Children's Hospital of Chicago. Each participant was consented to this IRB-approved study and screened with a REDCap survey for family history of ANS-related disorders, eye disease, and medications impacting the ANS. Only individuals lacking a family history of ANS-related disorders, eye disease, and not taking any medications affecting the ANS were offered inclusion in this study.

**Exclusion criteria** Participants < 2 years of age or > 21 years of age were excluded from analyses ( $N = 37$ ). Individuals taking medications that affect ANS functioning were not offered inclusion in this study [30].

### Study protocol

Participants were instructed not to consume caffeine-containing foods or beverages for 24 h prior to participation because of potential effects on the ANS. Participants were seated in a dark and quiet room for at least 1 min prior to pupillometry testing to establish a baseline pupil diameter. Pupillometry was conducted using the handheld NeurOptics PLR-2000 (Irvine, CA) with a fixed white light stimulus that flashed at 180  $\mu$ W for 31 ms. Measurements were taken monocularly over the course of 5 s at 0.33 s intervals. There was at least 1 min of rest between the tests on each eye. The device tests the PLR monocularly, so participants were counterbalanced on which eye was measured first ( $L = 130$ ,  $R = 189$ ). There were no significant differences in PLR variables by eye tested first ( $ps > 0.05$ ). Recording was obtained from each eye with values generated for each of the following sub-components of the PLR including the following variables: baseline pupil diameter

(PD) (mm; INIT; maximum PD), PD after light stimulus (mm; END; minimum PD), % pupillary constriction (DELTA; this is the percent decrease in pupil diameter after light presentation, thus a larger negative value indicates greater change—it is calculated as  $[(\text{INIT}-\text{END})/\text{INIT}]*100$ ), latency prior to constriction (s; LAT), average constriction velocity (mm/s; ACV), maximum constriction velocity (mm/s; MCV), average dilation velocity (mm/s; ADV), and time from light stimulus to when the eye reached 75% of INIT PD (s; T75).

### Data analysis strategy

Analyses were conducted on seven of the eight aforementioned variables. The variable T75 was analyzed as an exploratory variable of interest because 167 of 646 observations did not generate a T75 value, indicating that 25.8% of eyes tested did not re-dilate to 75% of baseline pupil diameter (INIT PD). Measurements from the right and left eyes were averaged to create mean responses for each of the PLR variables, yielding a maximum of 323 observations. The average was used in all subsequent analyses except on the exploratory data analyses regarding the T75 variable, given the intra-participant variability in obtaining a T75 variable.

*Analysis of variance (ANOVA)* Analyses using repeated measures ANOVAs with the age bins stipulated by the Eunice Kennedy Shriver National Institute of Child Health and Human Development were conducted to examine if there were specific age groups that demonstrate significantly different values for the PLR variables that may provide benchmarks for practicing clinicians and researchers [31]. These age groups for analyses were as follows: (1) early childhood, 2–5 years, (2) middle childhood, 6–11 years, (3) early adolescence, 12–18 years, and (4) late adolescence, 19–21 years. Repeated measures ANOVAs were also conducted using sex as a fixed factor to explore interactions. Analyses were conducted using *R*, *R*'s base packages, and the nlme package [32, 33]. To examine differences between individuals who obtained a T75 variable and those who did not, a series of one-way ANOVAs was conducted. All post hoc comparisons were corrected using Tukey's honest significant difference (HSD) test.

*Non-linear analyses* To examine the hypothesis of age as a significant predictor for the pupillary light response, a series of regression analyses were conducted with age as the primary predictor. Furthermore, considering the non-linear development of the ANS over the course of the lifespan as evident by initial increased PD and subsequent decreased PD in older adulthood [7, 14, 34], a stepwise forward selection process with additional polynomial terms in the regression equations was utilized to determine the relationship between

age and PLR variables. Analyses focused on linear, quadratic, and cubic trends to assess change over time. Exploratory analyses involved adding sex as an additional predictor to the final regression model for each variable, given potential puberty effects [34].

## Results

### Study cohort

Healthy participants included in analysis totaled  $N = 323$  (646 eyes; 175 females). Mean age and standard deviation (SD) at testing were  $11.78 \pm 4.89$  years, with a range of 2.30–21.93 years. Racial and ethnic distribution of participants was 61.0% Caucasian, 14.9% Hispanic, 8.3% African American, 14.6% Asian, 1% Native American, and 0.2% unknown. Corrective lens use was reported in 34.4% of participants, though participants did not wear contact lenses or glasses at the time of pupillometry. Distribution of eye color was as follows: 25.7% blue; 73.7% brown, green, or hazel; and 0.4% unknown. Anisocoria, or an initial pupil diameter difference  $> 0.5$  mm, was identified in 19% of the participants. Specifically, anisocoria was present in 12.5% of individuals 2–5 years old, 18.5% of individuals 6–11 years old, 18.3% of individuals 12–18 years old, and 24% of individuals 19–21 years old. See the supplementary data set for all participant information by observation (Supplementary Material 1). Table 1 presents means and standard deviations by age group and sex for all observations collected.

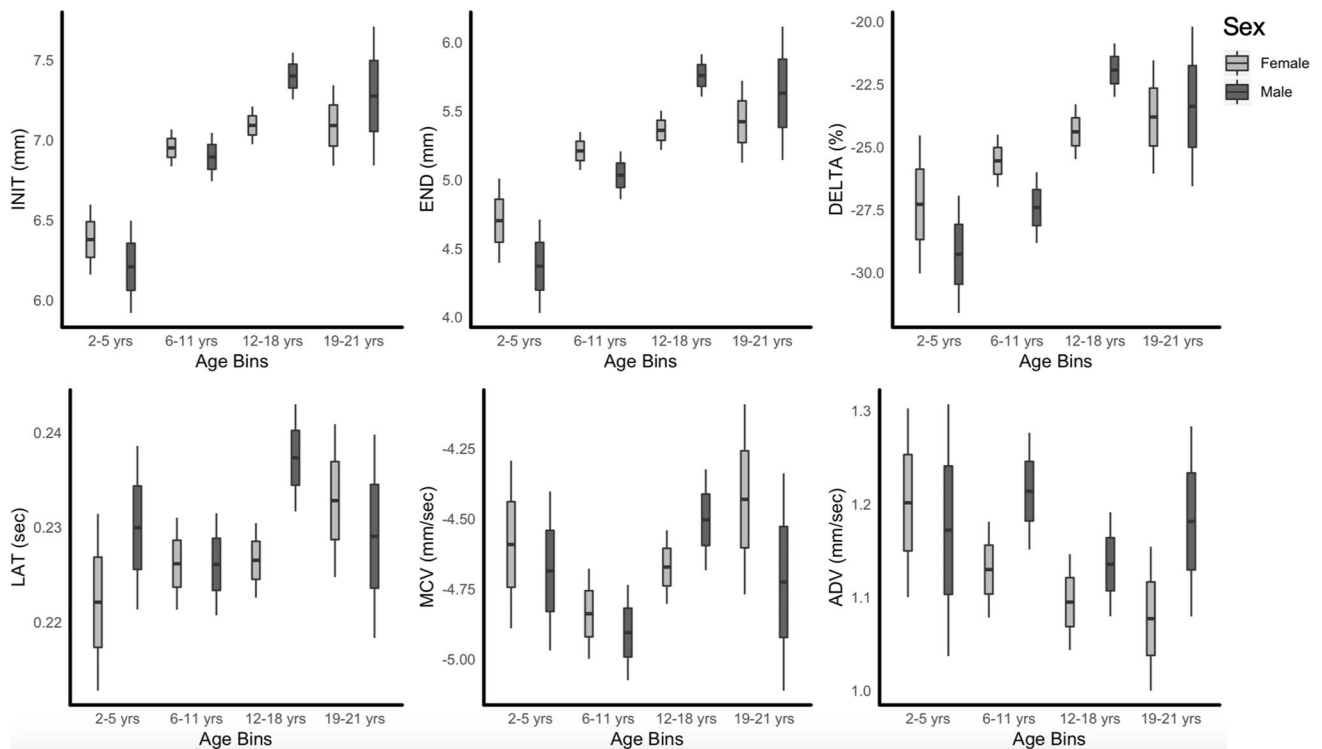
### ANOVA results

*Pupillometry measures by age group differences* (Fig. 2): Age-binned ANOVAs revealed age differences demonstrating that participants aged 12–18 years had a slower MCV than participants aged 6–11 years ( $p < 0.05$ ). Furthermore, children ages 6–11 years displayed a greater overall change in pupil diameter (DELTA) than children ages 12–18 years ( $ps < 0.01$ ). Children ages 2–5 years also had a significantly smaller initial and ending pupil diameter (INIT, END) than all other ages ( $ps < 0.001$ ). Similarly, children ages 6–11 years had a significantly smaller initial and ending pupil diameter (INIT, END) than children in early adolescence (12–18 years;  $p < 0.05$ ). No differences emerged between age bins for ACV, ADV, and LAT.

**Table 1** Pupillary light response values by age and sex in a healthy pediatric control cohort

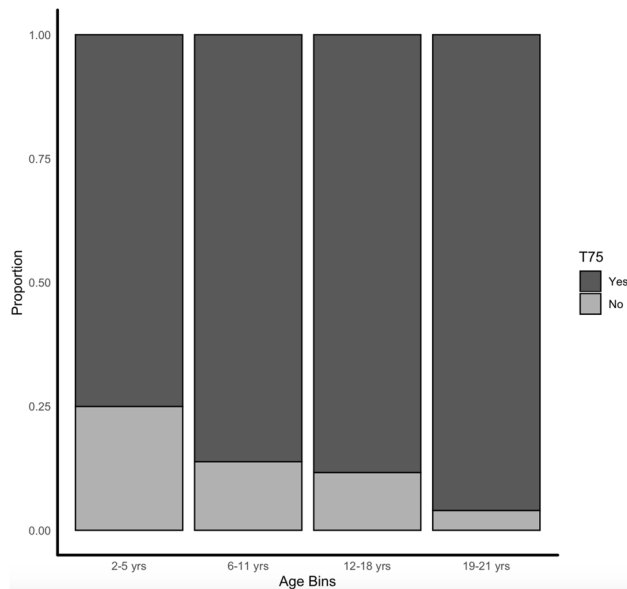
Sex <i>N</i> =number of eyes	Years	INIT (mm; M, SD)	END (mm; M, SD)	DELTA (%; M, SD)	LAT (s; M, SD)	ACV (mm/s; M, SD)	MCV (mm/s; M, SD)	ADV (mm/s; M, SD)
Female <i>N</i> =42	2–5	6.38 (0.72)	4.70 (1.01)	–27 (9)	0.22 (0.03)	–3.44 (0.70)	–4.59 (0.99)	1.20 (0.32)
Male <i>N</i> =30	2–5	6.21 (0.78)	4.37 (0.95)	–29 (6)	0.23 (0.02)	–3.39 (0.62)	–4.68 (0.79)	1.17 (0.37)
Female <i>N</i> =139	6–11	6.95 (0.69)	5.21 (0.83)	–25 (6)	0.23 (0.03)	–3.52 (0.74)	–4.84 (0.96)	1.13 (0.30)
Male <i>N</i> =117	6–11	6.90 (0.83)	5.04 (0.96)	–27 (8)	0.23 (0.03)	–3.58 (0.75)	–4.90 (0.93)	1.21 (0.33)
Female <i>N</i> =128	12–18	7.09 (0.67)	5.36 (0.82)	–24 (6)	0.23 (0.02)	–3.42 (0.62)	–4.67 (0.76)	1.10 (0.29)
Male <i>N</i> =110	12–18	7.40 (0.78)	5.76 (0.82)	–22 (6)	0.24 (0.03)	–3.30 (0.71)	–4.50 (0.96)	1.14 (0.30)
Female <i>N</i> =28	19–21	7.09 (0.68)	5.43 (0.80)	–24 (6)	0.23 (0.02)	–3.31 (0.69)	–4.43 (0.91)	1.08 (0.21)
Male <i>N</i> =22	19–21	7.28 (1.04)	5.63 (1.16)	–23 (8)	0.23 (0.03)	–3.58 (0.70)	–4.72 (0.93)	1.18 (0.24)

Mean (M) and standard deviation (SD) of each pupillometry variable including all observations for females and males in each age bin as defined by the Eunice Kennedy Shriver National Institute of Child Health and Human Development. INIT refers to the baseline pupil diameter. END refers to the pupil diameter after the light stimulus. DELTA refers to the percent decrease in pupil diameter after light presentation, with larger negative values indicating greater change. LAT is the latency to initiating a light response after the light stimulus. ACV, MCV, and ADV refer to the average and maximum constriction velocities, and average dilation velocity, respectively



**Fig. 2** Averages calculated for each participant in each age group by sex for INIT, END, DELTA, LAT, MCV, and ADV. Age bins are defined by the Eunice Kennedy Shriver National Institute of Child Health and Human Development. Light gray bars indicate data for

females, and dark gray bars indicate data for males. The central horizontal black lines indicate mean values, whereas the top and bottom horizontal black lines indicate  $\pm 1$  SD. The vertical black lines indicate  $\pm 2$  SD



**Fig. 3** Proportion of observations that received a T75 value (time in ms until the pupil reached 75% of its initial diameter) in each age bin as defined by the Eunice Kennedy Shriver National Institute of Child Health and Human Development

in sex distribution (54% females). Furthermore, participants who had a T75 value had a smaller INIT ( $p=0.006$ ), more robust ACV and MCV ( $p < 0.01$ ), and greater ADV ( $p < 0.01$ ), but no differences between groups when comparing END, DELTA, or LAT ( $ps > 0.05$ ).

### Non-linear regression results

*Pupillometry measures by age* (Table 2) The model of best fit exploring PLR function by age in our cross-sectional sample was determined by a stepwise forward selection model for each polynomial term: linear, quadratic, or cubic. For DELTA and ADV, age was linearly associated with pupil change and dilation velocity ( $ps < 0.05$ ). For INIT and END, a stepwise forward selection process adding polynomial terms in the regression equations revealed age as a significant predictor for both PLR variables using a second-order (quadratic) polynomial equation ( $ps < 0.05$ ). Finally, ACV and MCV were best modeled using a third-order (cubic) polynomial regression equation, though the third-order polynomial term was marginally significant for ACV ( $p=0.05$ ). Age was not a significant predictor of LAT ( $p=0.53$ ).

**Table 2** Polynomial regression equation by age for each pupillary light response variable in a healthy pediatric control cohort

Polynomial term	DELTA Estimate, SE	ADV Estimate, SE	INIT Estimate, SE	END Estimate, SE	ACV Estimate, SE	MCV Estimate, SE
Linear	0.276, 0.086	-0.008, 0.003	0.325, 0.070	0.169, 0.053	-0.359, 0.123	-0.516, 0.165
Quadratic	-	-	-0.010, 0.003	-0.004, 0.002	0.030, 0.011	0.043, 0.015
Cubic	-	-	-	-	-0.001, 0.000	-0.001, 0.000

Estimates and standard errors (SE) for the polynomial regression models derived for each pupillometry variable to assess change over time from all participants 2–21 years old. DELTA refers to the percent decrease in pupil diameter after light presentation, with larger negative values indicating greater change. ADV refers to the average re-dilation velocity. INIT refers to the baseline pupil diameter. END refers to the pupil diameter after the light stimulus. ACV and MCV refer to the average and maximum constriction velocities

*Pupillometry measures by age and sex* (Fig. 2): Across INIT and END, differences were evident in males aged 12–18 years such that they demonstrated the largest pupil diameter compared with all younger participants, male and female ( $ps < 0.05$ ). Similarly, the differences seen in DELTA were driven by males aged 12–18 years, who demonstrated a decreased DELTA compared with males and females aged 6–11 years ( $p < 0.05$ ). No other sex differences or age by sex interactions emerged in the other PLR variables.

*T75* Given the number of observations without measurable T75 ( $N=167$ ; 25.8%), we investigated potential reasons for the disparity. Descriptively, there was a greater percentage of participants in the early childhood range who did not have a T75 measurement (40%) compared with those who did not in the middle childhood (22%), early adolescence (26%), and late adolescence (18%) age bins (Fig. 3), but no difference

*Pupillometry measures by age and sex* When adding sex as an exploratory predictor to the model of best fit for each PLR variable, there were no significant effects of sex on any PLR variable ( $ps > 0.05$ ).

### Discussion

The present study details the largest prospective application of quantitative pupillometry in a healthy pediatric cohort examining age and sex using linear and non-linear models, with the aim to offer normative values for comparison with disease states, opiate exposure, and concussion. Results of the current study support the hypothesis that the components of the PLR change with age determined by linear and non-linear analyses. Aspects of the PLR seem to mature

according to different polynomial functions, reflecting dynamic patterns and rates of autonomic maturation with advancing age. Our findings suggest that ANS maturation and PLR regulation are dynamic and that in future research and clinical work chronologic age should be factored into understanding of normative PLR functioning. This study establishes normative values for the PLR as well as the prevalence of anisocoria (which is commensurate with previous literature [35]) in a pediatric population (2–21 years) that can be used as a benchmark in future evaluations of the PLR as a biomarker for autonomic regulation in health and disease.

Previously, it was posited that DELTA and LAT variables would be most associated with age. Though this has yet to be supported by empirical research [5], the present study demonstrated that DELTA decreased with advancing age in our cross-sectional sample according to a linear function, despite the lack of differences within age bins, though age was not a significant predictor of LAT. Descriptively, T75 values (reflecting re-dilation of the pupil) also increased in prevalence with age. Regarding the overall trend of maturation, it has been previously hypothesized that measures responding to the light stimulus mature because of the ongoing myelination that occurs throughout the lifespan [5]. The present study partially supports the idea that myelination contributes to the effect age has on PLR because age is primarily predictive of dynamic variables (MCV, ACV, ADV) that depend upon neurotransmitter signaling. However, results suggesting that DELTA seems to decrease with advancing age may imply that myelination is not the sole reason for the relationship between age and the maturation of the PLR. Given the sporadic sex differences revealed in the ANOVA analyses in the current study, it is hypothesized that components of the PLR may be uniquely affected by puberty-related hormones [36].

Both INIT and END pupil diameters demonstrated a quadratic pattern of change over time, which is consistent with our hypothesis of a non-linear pattern. Interestingly, males aged 12–18 years demonstrated a unique increase in pupil diameter compared with other age-binned groups. This is consistent with previous research detailing that the INIT and END pupil diameters decrease with age in older adulthood [7, 15, 37], though this specific increase in 12–18-year-old-males has not been previously reported. We also descriptively observed INIT decreasing after 18 years of age, suggesting a potential relationship with puberty in males. A large-scale retrospective review conducted on 1306 participants aged 0–17 years also demonstrated an overall increased pupil diameter [35] but using a different analytical method, and the sample did not extend to old enough subjects to see the subsequent decrease documented in our study. A quadratic pattern as a function of age for initial pupil diameter was reported among 1311 individuals aged

0–19 years collected over 20 years, though a different pupillometry technique was utilized [38]. Decreased initial pupil diameter with advancing age has been hypothesized to be associated with decreased SNS activity [7, 37]. This pattern of increasing pupil diameter in childhood followed by decreasing pupil diameter in adulthood could result from the following two processes: (1) maturation of the sympathetic nervous system in early childhood, which leads to increasing baseline pupil diameter, and (2) a decrease in sympathetic nervous system tone coupled with increased PSNS drive in adulthood.

Regarding velocity terms (ACV, MCV, and ADV), we observed a non-linear cubic maturation pattern as a function of age for ACV and MCV, whereas we observed a linear association with ADV. Prior literature suggested weak correlations between these variables, but did not apply non-linear patterns of maturation to the analysis. Regarding maturation of the ANS, it is important to account for potential non-linear changes given known non-linear development of other biologic processes. This is the first study that investigated PLR variables using polynomial terms, establishing a non-linear relationship between age and maturation of the PLR. We hypothesize that this non-linear relationship may be modulated by a number of processes such as neuroendocrine control, hormonal changes and pubertal status, ongoing myelination, and neurotransmitter prevalence. Additional research is necessary to further understand the relationship between the aforementioned variables and maturation of the PLR.

Though the results of this research are compelling, we have identified limitations to be considered in interpreting the data. Our study was conducted cross-sectionally instead of longitudinally, which prevents us from clarifying how the PLR may mature within the population over time. Moreover, in our cross-sectional sample, we had fewer participants in the early childhood and late adolescent categories, which may have impacted our ability to detect differences when compared with our larger age groups. Finally, our study did not investigate the test-re-test reliability of the pupillometer. Future research may benefit from conducting a longitudinal study of the PLR or, if done cross-sectionally, should take care to over-sample participants in the younger and older age bins. Furthermore, to support the use of pupillometry in a clinical setting, it will be important to examine the test-re-test reliability of pupillometer devices.

Overall, our results indicate that the PLR seems to mature with age, so it is important that clinicians appreciate that normative values will differ depending on the age of the individual being tested and the methodology used for assessment. The normative values in this PLR study provide clinicians with benchmarks for comparison when using the PLR *NeuroOptics-2000* following the same protocol put forth in this study, allowing them to

determine quantitatively how much their patient's measurements deviate from an expected norm. This normative cohort serves as a comparison tool that will aid clinicians in understanding each patient's baseline autonomic tone, which is invaluable for the understanding of changes with illness or disease and monitoring for changes with pharmacologic intervention, especially in the case of autonomic disorders/dysregulation. Moreover, the use of pupillometry in critical care, particularly in the use of monitoring brain injury severity, is growing [25, 27–29]. The PLR is also being examined as a biomarker in neurodevelopmental disorders, psychiatric disorders, and a variety of autonomic conditions [18, 19, 23, 24, 39] and as a reliable biomarker for opiate use [40]. Perhaps the most promising opportunity will be comparison of baseline pupillometry measures (obtained during a well-child visit) to post-concussion or post-opiate pupillometry, serving as a non-invasive guide to acute intervention and long-term follow-up.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** The protocol of this study was approved by the Ann & Robert H. Lurie Children's Hospital of Chicago's Institutional Review Board and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All participants gave their informed consent or assent prior to their inclusion in the study. Participants who provided assent were consented by their legal guardian.

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