



# Pseudo normative pattern electroretinograms in young children and infants

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## Abstract

**Purpose** Review Pattern Electroretinogram (PERG) data from a pediatric population to characterize the development of response.

**Methods** A case review of 104 subjects who had PERG aged between 0 and 9 years of age as part of routine clinical testing who were categorized as normal. PERG responses were recorded with skin electrodes, with the stimulus presented on a 40° screen. The main outcome measures were the amplitude and timing of the main components of the PERG, the P50 and N95.

**Results** An increase in P50 amplitude was noted over the first 2 years of life, a less rapid increase in amplitude was observed after this. The P50 timing was noted to decrease in the first 6 months but stabilized after this. A similar increase in amplitude was observed for the N95 amplitude with a modest decrease in latency.

**Conclusion** This study has shown an increase in PERG amplitude beyond the first 6 months of life with the most rapid rate of increase of response occurring in the first 2 years. This physiological data potentially documents the functional maturation of the human macula that parallels the anatomical changes noted via histology.

## Key messages

- Robust PERG responses are possible in pediatric cases recorded with skin electrode to widefield stimuli, even in young infants.
- Paper provides evidence of physiological development of macula that correlates well with previously documented anatomical maturation.

**Keywords** Pattern electroretinogram · PERG · Macular function · Maturation · Pediatric · Case series

## Introduction

There is important development of central vision in the first few years of life, with estimates of foveal maturation ranging from 11 months to 5 years on histological evidence [1–3], Ocular coherence tomography (OCT) suggests anatomical development of the retina continues until adolescence [4]. Psychophysical testing suggests visual function continues

into adulthood [4, 5] (likely due to maturation of visual cortex). There appears a lack of objective physiological evidence documenting the maturation of the macular. Whilst there is a considerable body of work exploring the pattern reversal visual evoked potential (PRVEP) [6, 7] relatively little work has been carried out investigating the physiological development of the macular. The pattern electroretinogram (PERG) measures the bio-electrical activity generated from the retina which can in turn be used as a bio-marker of macular physiology or function [8].

Recording the PERG can be technically challenging, as measuring such a small potential with a relatively massive standing potential between the front and back of eye in such proximity, can result in eye movement artefact which could cause bioelectrical drift in recording [9, 10]. Despite the PERG being first described by Riggs in 1964 [11], its popularity clinically only took off with the advent of corneal electrodes that did not obstruct the optics of the eye [10, 12,

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13], but still yielded relatively large signal direct from the cornea/sclera.

With infants and young children this may prove yet more challenging with many reluctant to have an 'electrode in the eye' or if willing may find it too uncomfortable not to blink. Kriss demonstrated that despite smaller signals a more pediatric friendly skin electrode [14], could provide important clues in the management of eye disease in children [15, 16] in the full field ERG.

McCulloch demonstrated a healthy sized (P50 ~ 5uV) PERG could be recovered using skin electrode with the aid of a widefield stimulator approx. 28° x 22° with checks of 40' width [17]. Others have noted a larger response to widefield stimuli [18, 19] which may mitigate against signal loss by not using a corneal/scleral electrode. A widefield stimulus has been used with skin electrodes in a paradigm to detect glaucoma [20].

Until recently ISCEV (international Society of Clinical Electrophysiology of Vision) insisted recording be made via corneal electrode [21] with skin electrodes only used in circumstances where the corneal electrode cannot be tolerated, i.e. with pediatrics and then reported with caution [10], and with the recommendation of prolonged averaging (to improve Signal to Noise Ratio (SNR) [22]. Animal studies have shown whilst the response is macular driven [23], it is the ganglion cells that generate the N95 and much of the P50, though the proportion of contribution may differ slightly across species [8, 23] and likely again in man. Clinically when there is insult to the ganglion cell the P50 is often reduced and early, with N95 reduction and N95/P50 ratio reduction [10, 24–26].

This paper provides robust evidence that with the use of widefield stimuli PERGs can be recorded in infants and young children. Our findings indicate a modest increase in signal amplitude in the early months without gross change in timing which compliments the anatomical/histopathological work of Hendrickson [2, 3]. These findings came from a cohort of patients seen at Children's Hospital of Pittsburgh who were categorized as normal after a complete ophthalmic assessment with no changes on funduscopy, with good PRVEP when seen in our laboratory and if recordable good visual acuity (20/30 or better).

## Methods

**Study design** Retrospective case-note review of all children who underwent PERG recordings employing skin electrodes and a widefield pattern stimulator attending the Children's hospital of Pittsburgh between January and December 2021. Institutional Review Board (IRB)/Ethics

Committee approval was obtained with a waiver of consent, given its retrospective nature.

**Patients** Potential subjects were identified via the visual electrophysiology database. Subjects were omitted if they had abnormal clinical examination (fundus/optic nerve), were suspected of reduced vision function, could not comply with the test, or fixation on stimulus was deemed poor and testing abandoned. The children included had no history of visual or neurological diseases, or head trauma with unconsciousness. Subjects had a minimum corrected acuity of 20/30 or in younger subjects a normal PRVEP to 25' for under 1 year and 12' for over 1 year. It was not possible to follow up all children, as many, once concerns regarding poor visual behavior were allayed were discharged.

A total of 104 participants were identified and included in the study (42 girls and 62 boys). All subjects underwent PERG recordings with both eyes open. Testing was conducted with natural pupils using current correction were necessary. Testing was conducted in the dark to avoid distraction and help focus on the stimuli/cartoon/movie. The stimuli were presented on a Pioneer© plasma screen consisting of a high contrast black and white reversing checkerboard of 50' in size with a mean screen luminance of 91 cd/m<sup>2</sup> and 95% Michelson contrast at 1 m. The stimuli were presented at 2.5 reversals per second. The screen subtended a visual angle of 40° x 28°.

PERG were recorded employing silver-silver chloride disposable adhesive electrodes placed on the interior orbital rim of each eye just below the lower eyelid crease referenced to the outer canthus with matched skin impedance of 5kΩ or below. Data was collected at a sampling rate of 1 kHz and a band-pass filtered (0.312–100 Hz) before being saved. Recording epochs were 285ms in duration including 10ms pre-stimulus. The amplifiers had a fixed gain with an input range of +/-0.5 V (Espion by Diagnosys LLC, Lowell, MA, United States). Any epochs with activity exceeding ± 200 uV were automatically rejected during data acquisition. Two reproducible trials were recorded with each trial comprising at least 50 epochs before being averaged to be used for data analysis. Post-hoc 'cleaning' of data was performed, removing any individual runs of obvious slew utilizing the pre-stimulus portion of the recording, or if excessively noisy due to mains intrusion, EMG or eye movement. De-trending of traces with slew was not conducted, but simply excluded, though de-trending is now recommended by ISCEV [22].

During the recordings the subjects sat on their parent's lap 1 m from the screen and their fixation to the pattern stimuli

**Table 1** Amplitude and time to peak of P50 and N95 for the various age groups. N=number of participants

Age months	P50 uV (±SD)	N95 uV (±SD)	P50 ms (±SD)	N95 ms (±SD)	N=
0 to <6	3.3 (0.9)	4.6 (1.4)	50.9 (2.9)	105 (8.1)	38
6 to <12	3.7 (1.0)	5.9 (1.1)	50.0 (2.2)	99.7 (5.8)	24
>12 to 24	3.4 (1.0)	5.2 (1.5)	49.0 (1.9)	97.6 (2.9)	30
>24 to 36	3.9 (1.2)	6.0 (1.6)	48.4 (1.8)	96.3 (3.6)	28
>36 to 60	4.1 (1.0)	6.3 (1.7)	48.4 (1.6)	95.0 (3.3)	30
>60 to 84	4.3 (1.2)	6.4 (1.6)	47.8 (2.2)	95.4 (3.7)	30
>84 to 108	4.8 (1.3)	7.1 (1.6)	47.2 (1.8)	94.7 (3.2)	28

was monitored via closed circuit television, enabling recordings to be paused if attention deviated from the stimuli. A DVD chosen by the subject's parent was viewed between stimulus presentation periodically to maintain the child's attention during the recording. Throughout the recordings the audio track was audible to maintain alertness.

**Data analysis** The amplitude of the major PERG components were measured for each eye and tabulated for analysis. Subjects were grouped depending on age, age range were shorter when younger as this provided a more detailed description during the period of the most rapid change, but also in our lab we tend to see more infants and young children so by doing this group size was more comparable. The PERG N35-P50 and P50-N95 amplitude was measured in addition to the P50 and N95 time to peak. In subjects with poorly defined N35, baseline or a negativity between 20 and 40ms was taken as the reference point for P50 amplitude, whichever gave largest response. Cardinal peaks were identified by software and verified by an experienced visual electrophysiologist. Statistical analysis

(curvilinear regressions) was carried out employing IBM SPSS statistics version 28.

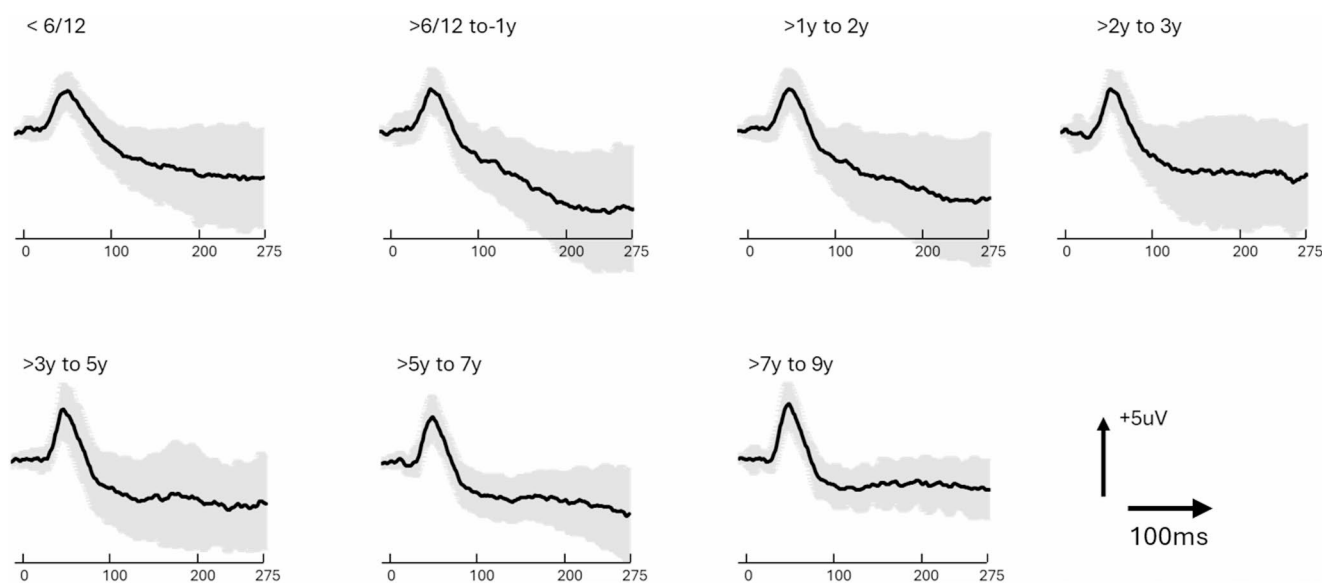
The amplitude and time to the peak of PERG components were compared between age groups (see Table 1) using the non-parametric Kruskal-Wallis Test tests with any significant interactions investigated employing Bonferroni adjusted Dunn's pairwise tests.

## Results

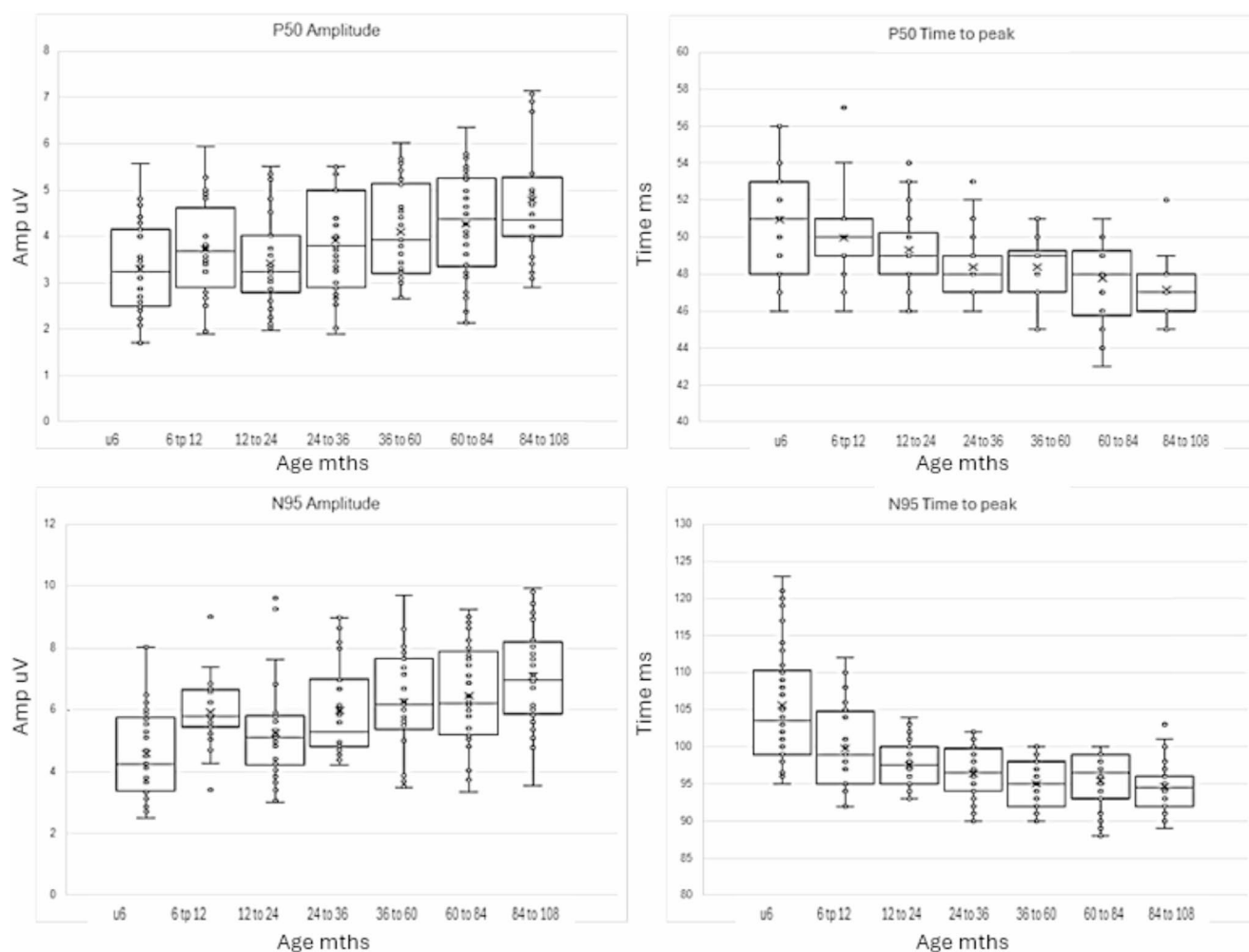
Measurable PERGs were recorded in all participants. All responses were adult-like in shape consisting of a positivity around 50ms followed by a negativity in the 95ms range (Fig. 1). For further analysis responses were grouped by age: Under 6/12, <6/12 to 1 year, <1 to 2 years, <2 to 3 years, <3 to 5 years, <5 to 7 years and <7 to 9 years. The mean P50 and N95 values are presented below in Fig. 2; Table 1.

The Kruskal-Wallis tests indicated that there was an effect of age group on the P50 ( $\chi^2 [6], N = [208] = 31.49, p < 0.001$ ) and N95 ( $\chi^2 [6], N = [208] = 46.26, p < 0.001$ ) amplitude, and P50 ( $\chi^2 [6], N = [208] = 48.97, p < 0.001$ ) and N95 ( $\chi^2 [6], N = [208] = 46.26, p < 0.001$ ) time to peak.

The findings of Dunn's pairwise tests (adjusted using the Bonferroni correction) are presented in Table 2. Only statistically significant differences are presented. The P50 and N95 amplitude were found to be smaller in younger participants compared to older ones while the time to peak was longer in younger participants compared to older ones.



**Fig. 1** Group average waveforms for the various age groups. Grey shaded area=standard deviation



**Fig. 2** Mean amplitude and time to peak of P50 and N95 for different age groups in months

The P50 amplitude is statistically larger between 5 and 7 years compared to under 6 months and on average was close to a third larger. The 7–9 years tend to be around one and a half times as large as the under 6 months group and this too was statistically significant.

The group between 1 and 2 years is not statistically different compared to 6–12 months and when compared to 2–3 years group, so any average difference could be due to noise.

N95 was larger in all groups compared to under 6 months except 1–2 years. The 5–7 group is on average slightly more than a third bigger than the under 6 months, whilst 7–9 group is on average slightly more than one and a half times larger than the under 6 months.

N95 responses tended to be 10ms on average earlier after 7 years compared to 6 months and under. The average N95 responses time to peak tended to be 5ms earlier after 7 years of age compared to the average of the 6–12 month group. The N95 tended to become better defined with age in this cohort.

## Discussion

This study has shown that the PERG can be robustly recorded using skin electrodes in infants and young children, with recognizable responses seen individually and in group averages. The P50 and N95 amplitudes in this group of patients with clinically normal fundus appearance, visual acuity and/or PRVEP were found to increase significantly with age. From the curvilinear graphs (Fig. 3) it appears this increase is most marked in the first 2 years of life. This correlates well with the histological findings of Hendrickson [2, 3]. Her work mostly focused on the morphological change of the fovea and photoreceptor density, whilst responses in this study are likely to originate from a larger area including macular and paramacular areas due to the size of the wide-field stimuli. Hendrickson has shown via histological data that the fovea starts to develop at 22 weeks gestation, but its morphology and cone density continue to develop up until 3 or 4 years of age [2, 3]. Foveal photoreceptor density was

**Table 2** Dunn's pairwise tests (adjusted using the Bonferroni correction)

<b>P50 amplitude (uV)</b>		<b>N95 amplitude (uV)</b>	
Pair comparisons	P =	Pair comparisons	p=
(<6/12) - (>5 to 7)	0.009	(<6/12) - (>6/12 to 1)	0.021
(>6/12) - (>7 to 9)	<0.001	(<6/12) - (>3 to 5)	0.001
(>1 to 2) - (>7 to 9)	0.002	(<6/12) - (>5 to 7)	<0.001
		(<6/12) - (>7 to 9)	<0.001
		(>1 to 2) - (>7 to 9)	<0.001

<b>P50 time to peak (ms)</b>		<b>N95 time to peak (ms)</b>	
Pair comparisons	p=	Pair comparisons	p=
(>7 to 9) - (>1 to 2)	0.004	(>7 to 9) - (>6/12 to 1)	0.016
(>7 to 9) - (>6/12 to 1)	<0.001	(>7 to 9) - (>6/12)	<0.001
(>7 to 9) - (<6/12)	<0.001	(>3 to 5) - (>6/12 to 1)	0.050
(>5 to 7) - (>6/12 to 1)	0.034	(>3 to 5) - (>6/12)	<0.001
(>5 to 7) - (<6/12)	0.001	(>5 to 7) - (>6/12)	<0.001
(>2 to 3) - (<6/12)	0.004	(>2 to 3) - (>6/12)	<0.001
(>3 to 5) - (<6/12)	0.024	(>1 to 2) - (>6/12)	0.004

found to increase in these early years measuring 36 thousand per mm<sup>2</sup> at birth, increasing to 53 thousand per mm<sup>2</sup> by 15 months and reaching lower limit adult levels of 103 thousand per mm<sup>2</sup> by 3.8 years (adult density 208 thousand per mm<sup>2</sup>) [1].

Ocular coherence tomography data has also shown macular development in these early years. Gottlob's group found central macular thickness increased logarithmically in the first 4 years of life [4]; while Toth's group noted a shallow foveal pit, and thin photoreceptor layer that was thinnest at the fovea [27].

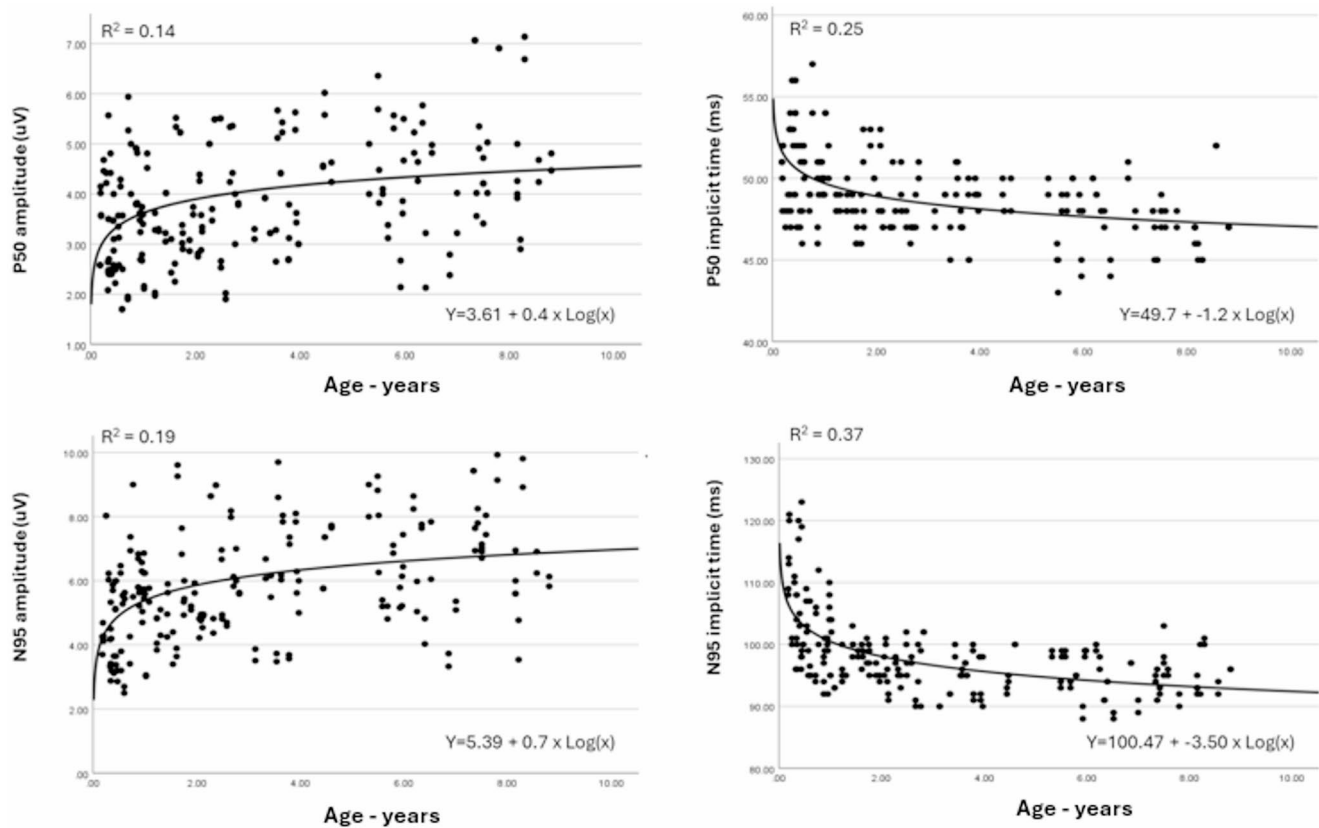
Full field Electroretinograms (ERG) have been shown to mature also, at 10 weeks, infants' sensitivity and saturated cone amplitude response are still developing measuring approximately 68% and 72% that of adult's response [28].

There appears little work on the maturation of PERG (physiological development of the human macular) and what has been published is not in full agreement. Odom [29] found in three subjects aged between 2 months and 4 months (using DTL thread and a wide screen projection approximately 32° by 20°) that there was not a marked difference with adults, with both cohorts faring better to larger

checks. In contrast Fiorentini [30] found a marked decrease in timing of the main positivity in the first 5 months in her 10 subjects, who were recorded on average twice between the ages of 4 weeks and 24 weeks.

The current study found only a modest decrease in P50 and N95 latency, though testing was performed at different, luminance, stimulation rate and using a different check size compared to Fiorentini [30]. The current study ensured reproducibility of responses by having at least two reproducible averages containing an average of 160 runs. There appeared to be good correlation of responses intra and inter age groups with easily recognizable group averaged PERGs recorded across all age groups.

Like Odom [29] response morphology and timing were not grossly different between infants and older children in our cohort, however a modest statistically significant difference in amplitude and timing was seen between our youngest and oldest children. The N95 also appeared to become better defined in the older group (indeed the current ISCEV standard suggests not reporting the N95 timing due to variability [22]). Although a confounding factor may be co-operation, the setup in Pittsburgh is based on the Kriss



**Fig. 3** Graphs showing the logarithmic curve fit between amplitude/implicit timing and age in years

laboratory and ethos [16] with a large screen playing the child's favorite cartoons, with skilled technicians engaging the children and CCTV to monitor fixation.

Infants usually comply well though between 18 months and 3 years of age, testing was at times a greater challenge. Other possible unknowns are the impact of developing optics on the responses and the makeup of skin impedance of very young infants and whether this might impact the recording characteristics of the skin electrode.

Vaegan's group noted an increase in response with field size in a logarithmic fashion [18] as did Lenassi [31]. So, whilst the area of field is width  $\times$  height, the response does not increase quite linearly with area or with field width but there is a substantial gain in signal by doubling the field width (quadrupling the test area, when aspect ratio is maintained). Thompson's group found the P50 almost trebling in size when the field width was doubled from  $15^\circ$  to  $30^\circ$  recording with a thread electrode [19]. They also found a small increase in amplitude when recording in the dark amounting to an increase of around one fifth of the amplitude to  $30^\circ$  stimulation in the light, under these conditions on average they recorded 8.2  $\mu$ V when using thread electrode in

their cohort of 21 healthy adults [19]. The widefield stimulus makes it possible to record robust responses with skin electrodes as previously described by McCulloch (McCulloch et al., 1998). Furthermore, we would agree with McCulloch that not placing an electrode in the eye would likely reduce the need for blinks, causing artefacts and drift, which make PERG challenging to acquire as noted by others [9, 10, 17].

A limitation of the work is that we have drawn data from subjects requiring clinical testing and not healthy volunteers. At time of testing fundal examination was normal as was visual acuity, when possible, if this was not possible to perform, a normal PRVEP to small check was taken in its place. In these early years it may be difficult to recruit healthy volunteers willing to be tested and have cycloplegic examination. It seems reasonable to use data already recorded in subjects with normal eye exam to help establish likely maturation trends.

The PERG has an important role in determining post retinal dysfunction in conjunction with PRVEP [10, 24, 31–34] and this paper demonstrates that not only can this be collected non-invasively with skin electrodes, but in young infants utilizing a widefield screen.

## Conclusion

This paper confirms robust PERG can be acquired with skin electrodes when utilizing a widefield pattern stimulator in infants. The results collected in this cohort tended to show an increase in amplitude over the first 6 years but particularly in the first 2 years, which coincides with a marked change in foveal development noted histological and on OCT examination of neonates [1–4, 27]. We did not find a gross shortening of response timing in contrast to the findings of Fiorentini [30]. This study provides an insight into the physiological development of the macular complementing anatomical development.

The PERG is a biomarker for the function of the macular pathway, and this study not only demonstrates this method can be useful in infants and young children but provides information on the maturation of the response aiding clinical interpretation and facilitating understanding of the physiological development of the human infant macula. A limitation of this work is data is not taken from normal volunteers, but subjects requiring electrophysiology testing with normal fundi and visual acuity (were possible) or else small check PRVEP.

**Author contributions** Authors AL & KKN made substantial contributions to the conception or design of the work; Author AL made substantial contribution to the acquisition, Author AL & RPH analysis and interpretation of data; AL, RPH & KKN Authors drafted the work or revised it critically for important intellectual content; AL, RPH & KKN Authors approved the version to be published; and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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**Data availability** The data that support the findings of this study are not openly available due to patient confidentiality, though an anonymized summary of amplitudes, timing with age range is available upon reasonable request. Data are located in controlled data storage at University of Pittsburgh Medical Centre.

## Declarations

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (University of Pittsburgh IRB) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

This was a retrospective case series review and not a clinical trial. IRB study number 21020113.

**Competing interests** The authors declare no competing interests.

**Conflict of interest** The authors have no relevant financial or non-financial interests to disclose.

**Clinical trial number** not applicable.

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