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BASIC SCIENCE

Association analyses of the measurements of the photopic negative response evoked by two ISCEV protocols

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Abstract

Purpose To perform association analyses between the measurements of photopic negative response (PhNR) evoked by two ISCEV protocols.

Methods A total of 172 eyes from 72 post-operative pediatric cataract patients and 24 healthy children were enrolled. The amplitude and peak time of PhNR were analyzed in three eye groups, 1. healthy controls; 2. fellow eyes of unilaterally affected patients; 3. affected eyes. PhNR responses were measured with skin-electrodes and evoked by the ISCEV standard protocols of PhNR and light-adapted 3.0, referred to as PhNR1 and PhNR2. The correlation coefficients between PhNR1 and PhNR2 measurements were calculated. The generalized estimating equation (GEE) model of PhNR1, with PhNR2 as a predictor, was evaluated after adjusting for correlation between paired eyes.

Results Both the amplitude ($P = 0.025$) and the peak time ($P = 0.036$) of PhNR1 showed a significant difference among the three eye groups, which was not observed in PhNR2. The four correlation coefficients (Pearson, Intraclass, Lin's and Kendall's) between z-score transformed PhNR1 and PhNR2 measurements were generally moderate: 0.52, 0.52, 0.52, 0.36 for amplitude ($P < 0.001$), and 0.57, 0.57, 0.57, 0.36 for peak time ($P < 0.001$). The amplitude of PhNR1 cannot be precisely predicted by PhNR2, with a mean absolute percentage error (MAPE) of 36.7%, while the peak time of PhNR1 can be precisely predicted with a MAPE of 3.9%.

Conclusions PhNR1 appears to be a more sensitive measure than PhNR2 for detecting eye group differences. Further research is needed to confirm this and explore its clinical applications. PhNR1 may not be entirely replaced by PhNR2 due to moderate correlation and low prediction precision in amplitude.

Key messages

What is known

- There are two main methods to evoke PhNR: the standard LA 3.0 ERG protocol and the ISCEV specialized protocol, but their comparability is unclear, affecting study consistency.

What is new

- In our study, the ISCEV specialized protocol may offer increased sensitivity in detecting differences among eye groups, but further research is needed to confirm this potential advantage.
- The two methods are not interchangeable due to moderate correlation and low prediction precision in amplitude.

Keywords Photopic negative response · Electroretinogram · Association analysis · Children

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Introduction

Electroretinogram (ERG) is the test which measures the electrical activity of the retina in response to light, which plays a vital role in the evaluation of retinal physiology and is widely applied in clinical practice and research. Photopic

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negative response (PhNR) is the negative-going potential after b-wave in light-adapted (LA) ERG, which reflects the activity of retinal ganglion cells and their axons [1, 2]. Over recent years, PhNR has gained interest in research as an important component of LA ERG, providing information on retinal ganglion cells.

There are two major different methods to evoke the PhNR. Firstly, as a component of LA ERG, PhNR can be measured via the standard protocol of LA 3.0 ERG, using a single white flash stimulus under white background luminance, according to the International Society for Clinical Electrophysiology of Vision (ISCEV) [3]. Meanwhile, the ISCEV also published a specialized protocol for measuring PhNR, which recommended using a blue background and red light flash [1], since the narrowband stimuli are reported to be more effective in eliciting PhNR response when compared to the broadband stimuli in LA 3.0 ERG [4, 5].

In clinical practice and research, some apply the LA 3.0 ERG protocol [6–8], while others use the ISCEV PhNR protocol [9, 10] to evoke PhNR. However, it remains unknown how the measurements of the PhNR responses by the two protocols agree with each other, which makes it unclear whether the outcomes are comparable between studies applying different protocols. On the other hand, if the two PhNR responses can predict each other precisely, it would be sufficient to perform only LA 3.0 ERG in participants with poor cooperation. This approach can save time and improve participants' comfort. However, to the best of our knowledge, there is a lack of such evidence.

In this study, we performed full-field ERG tests based on the protocol of LA 3.0 ERG [11], followed by ERG using the protocol for PhNR [1] in children. This study is based on data from a clinical study comparing the ERG of congenital cataract patients and healthy controls. The association analyses were conducted between the PhNR responses obtained by different protocols, stratified by three eye groups: the affected eyes, the fellow eyes, and the healthy eyes.

Methods

Study design and participants

From August 2020 to July 2022, this cross-sectional observation study was conducted at the Hangzhou Campus of the Eye Hospital of Wenzhou Medical University. Post-operative pediatric cataract patients and healthy children were enrolled in this study. For the patients, cataract removal and IOL implantation were performed before entering this study by the same surgeon (Y.Z) under general anesthesia with the Centurion Vision System (Alcon Co., USA). The study was approved by the ethics committee of the Eye Hospital of Wenzhou Medical University (Reference No. 2020–089-K-81–01). Written informed consent was provided by all participants' parents/guardians.

Measurements

ERG examinations

Mydriasis was performed before the ERG tests with Tropicamide Phenylephrine Eye drops (Santen Co., Ltd.), administered every 10 min for 3 times. ERG was conducted without sedation in all participants using the MonpackOne instrument (Metrovision, Perenchies, France), with both eyes of the same participant examined simultaneously. The skin electrodes (Model EEGW02, BaiEnHongTai, Qingdao, China) were used, with two active electrodes on the lower eyelids, two reference electrodes near the outer canthi, one ground electrode on the forehead.

The LA 3.0 ERG was performed before the LA ERG using the ISCEV standard protocols of PhNR [1, 11]. LA 3.0 ERG was performed after light adaptation. Under a background luminance of 30 $cd{\cdot}m^{-2}$, a single white flash stimulus was delivered for 5 ms with the strength of 3.0 $cd{\cdot}s{\cdot}m^{-2}$, and then the ERG responses were recorded by the electrodes. After a one-minute break under lighting conditions, the ERG test for PhNR was then performed. With a background luminance of blue light (wavelength, 465 nm; strength of 8 $cd{\cdot}m^{-2}$), a single red flash (wavelength, 619 nm; strength, 1.2 $cd{\cdot}s{\cdot}m^{-2}$) was delivered for 5 ms and then the ERG responses were recorded. The b-wave amplitude was measured from the baseline to the peak of the first positive deflection. The amplitude of PhNR was defined from the baseline to the maximum trough of the negative-going wave after the initial i-wave following the b-wave, and the peak time was defined as the corresponding time since stimulus onset [1]. Figure 1 illustrates the original traces and the measurement methods of these PhNR variables.

Other measurements

Intraocular pressure (IOP) was measured with an air-puff tonometer, Canon TX-20 (Canon Medical Systems, USA) in cooperative children and with the rebound tonometer icare PRO (Icare Finland Oy., Vantaa, Finland) in the other children. Axial length (AL) was measured with IOL-master 700 (Carl Zeiss AG, Oberkochen, Germany) before mydriasis, with the recording being an average of three reliable readings of good quality.

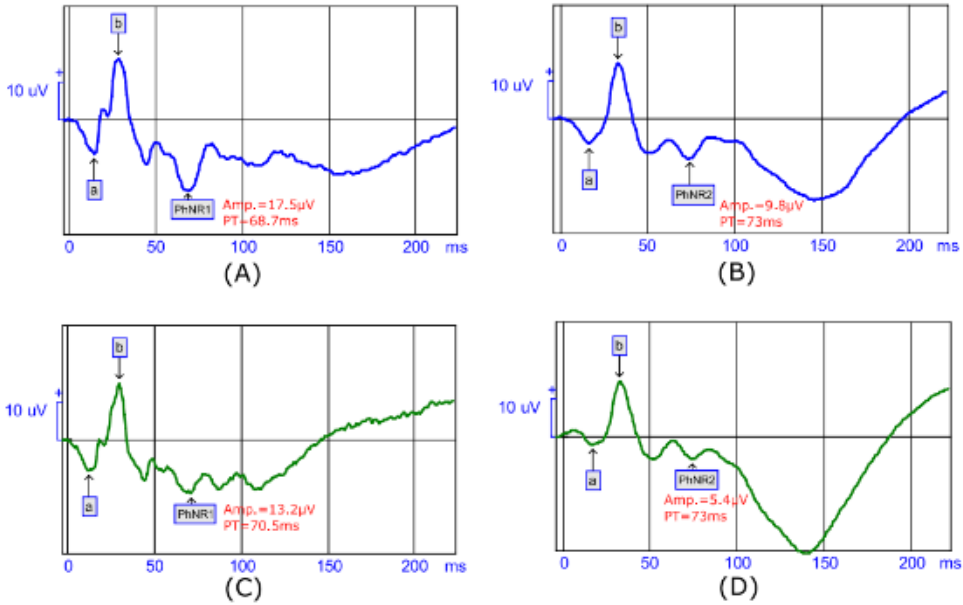

Fig. 1 The original traces of the healthy control eye (**A**, **B**) and the affected eye (**C**, **D**) from two participants aged 8 years old. **A**, **C** were evoked by the ISCEV extended protocol for PhNR, and (**B**, **D**) were evoked by the ISCEV standard protocol for LA 3.0 ERG

Eligibility

The eligible criteria and inclusion process of the participants are provided in S Fig. 1. We excluded patients who met any exclusion criteria: the participants who were younger than 3 years or those who had recent cataract removal (within 6 months) to rule out the effect of the surgery on ERG; the patients who were diagnosed with/suspected of glaucoma or ocular hypertension, or with other disorders as shown in S Fig. 1; whose parents/guardians were unwilling to participate this study; who resisted taking the ERG test; who took the ERG test but didn't cooperate well to finish it or with low ERG data quality. In the end, we excluded the eyes with undetermined PhNR waves in either ERG exam. Finally, a total of 172 eyes of 72 patients and 24 healthy children were enrolled for analysis.

Statistical analysis

The PhNR response to narrowband stimuli based on the ISCEV extended protocol for PhNR [1] was referred to as PhNR1, while the PhNR response evoked by the broadband stimuli based on the LA 3.0 ERG protocol [11] was referred to as PhNR2. Ratio1 was calculated as PhNR/b-wave amplitude based on the ISCEV extended protocol for PhNR [1], while Ratio2 was calculated as PhNR/b-wave amplitude based on the LA 3.0 ERG protocol [11]. The z-score was defined as the observed value minus the sample mean, divided by the standard deviation, which was used to calculate the correlation coefficients. The normality of the PhNR variables was tested with Q-Q plots. The test–retest reliability was assessed by treating measurements from the two eyes of the same participant as paired test and retest data. The reliability was evaluated using the Bland–Altman method and Cronbach's alpha coefficient in both healthy controls and bilaterally affected patients.

The enrolled eyes were analyzed in three groups, 1. healthy children's eyes as controls; 2. the fellow eyes of the unilateral patients; 3. the affected eyes of unilateral and bilateral patients. Age was calculated in days and analyzed as a continuous variable in years. In the descriptive analyses, given the skewed distributions of Ratio1 and Ratio2, we reported quartiles and used non-parametric tests. The Kruskal–Wallis test was used to compare the median values of the ratios across different eye groups. The paired Mann–Whitney test was used to compare the median values of Ratio1 and Ratio2 within the same individuals. For other variables, means and standard deviations of the continuous

variables were reported, and the generalized estimating equation (GEE) models were applied to adjust for correlation between paired eyes of the same patient and covariates [12].

In the association analyses, the z-score transformed amplitude and peak time were compared between PhNR1 and PhN2, and 4 different correlation coefficients were calculated, which included Pearson's correlation coefficient (PCC), intraclass correlation coefficient (ICC), Lin's concordance correlation coefficient (CCC) and Kendall's consistency coefficient (KCC) [13, 14]. The strength of the correlation coefficient was defined as, ≥ 0.80 very strong, 0.6 – 0.8 strong, 0.4 – 0.6 moderate, and < 0.4 weak.

The regression model of PhNR1, with PhNR2 measurement as a predictor, was built after adjusting for the correlation between paired eyes using GEE. Other covariates, including age, sex, IOP and AL, were selected according to the Bayesian information criteria (BIC). The regression models were then built, stratified by eye groups and evaluated with root-mean-square error (RMSE), mean absolute percentage error (MAPE), and the ratio of predictions within a given absolute percentage error (APE).

The Q-Q plots were generated using the R 4.3.2 (The R Foundation) and all other analyses were performed with STATA/SE (StataCorp LLC, College Station, TX). A Bonferroni-corrected significance level was applied for multiple comparisons, calculated as the significance level ($\alpha = 0.05$) divided by the times of comparisons.

Results

Demographics

As shown in S Fig. 1, a total of 172 eyes from 96 participants were enrolled for analysis, including 72 postoperative pediatric cataract patients (41 unilaterally /31 bilaterally affected) and 24 healthy children as control. As shown in Table 1, the mean age at baseline was 6.6 ± 2.2 years ($P = 0.040$ among the groups), and the proportion of males was 47% ($P = 0.732$). The mean age was highest in healthy controls (7.1 ± 1.0 years) and lowest in bilaterally affected patients (5.8 ± 2.3 years).

Comparisons of the measurements

Of all enrolled eyes, the mean AL was 22.8 ± 1.3 mm and the mean IOP was 15.3 ± 3.3 mmHg. After adjusting for age, sex and correlation between paired eyes with GEE, AL was significantly different among the three eye groups ($P < 0.001$), while no significant discrepancy in IOP was found ($P = 0.085$). Ratio1 (median 1.42, interquartile range 0.96–2.1) was significantly larger than Ratio2 (median 0.64, interquartile range 0.45–1.1; $P < 0.001$). However, neither ratio showed significant differences among the three eye groups ($P = 0.786$ and 0.111, respectively). For other PhNR measurements, the Q-Q plots indicate no obvious

Table 1 Descriptive analyses of the enrolled participants and eyes

Patient groups	Health children	Unilateral group		Bilateral group	Total	*P*-values	
N (%), participants	24	41		31	96	-	-
Age at exam (year)	7.1 (1.0)	6.9 (2.4)		5.8 (2.3)	6.6 (2.2)	0.04	-
Sex, male (%)	11 (46%)	21 (51%)		13 (42%)	45 (47%)	0.732	-
Eye groups	Control eyes	Fellow eyes		Affected eyes ($n = 89$)	Total eyes	*P*-values*	*P* for trend*
N (%), eyes	48	35	36	53	172	-	
Axial length (mm)	23.6 (0.8)	23.0 (1.1)		22.3 (1.4)	22.8 (1.3)	< 0.001	0.025
IOP (mmHg)	16.4 (3.6)	15.6 (3.5)		14.9 (3.1)	15.4 (3.3)	0.085	0.027
PhNR1 amp. (μV)†	15.2 (4.7)	13.1 (6.0)		11.8 (5.4)	13.0 (5.5)	0.025	0.053
PhNR1 peak time (ms)	69.7 (3.6)	69.6 (3.7)		68.7 (4.9)	69.2 (4.4)	0.036	0.055
PhNR2 amp. (μV)†	8.8 (4.0)	9.4 (4.5)		9.2 (5.2)	9.1 (4.7)	0.259	0.1
PhNR2 peak time (ms)	75.5 (3.2)	72.5 (3.4)		72.8 (5.3)	73.5 (4.6)	0.083	0.867
PhNR/b-wave Ratio						*P*-values**	
Ratio1‡	1.45 (1.14, 2.04)	1.49 (0.93, 2.6)		1.35 (0.95, 2.04)	1.42 (0.96, 2.1)	0.786	-
Ratio2‡	0.63 (0.39, 0.84)	0.65 (0.41, 1.1)		0.66 (0.48, 1.29)	0.64 (0.45, 1.1)	0.111	-

† PhNR1 = PhNR responses measured using the ISCEV extended protocol of PhNR, PhNR2 = PhNR responses measured using the ISCEV standard protocol for light-adapted 3.0 ERG

‡ Ratio1 = PhNR amplitude / b-wave amplitude (ISCEV extended protocol of PhNR), Ratio2 = PhNR amplitude / b-wave amplitude (ISCEV standard protocol for light-adapted 3.0 ERG); Ratio1 and Ratio2 were reported as medians (interquartile ranges)

* Adjusting for correlation between paired eyes, age and sex with generalized estimating equation model; for the PhNR variables, further adjusting for axial length and IOP

** *P*-values based on the Kruskal–Wallis test

skew distribution exists in the PhNR variables (S Fig. 2). After further adjusting for AL and IOP, both the amplitude ($P=0.025$) and the peak time ($P=0.036$) of PhNR1 showed significant differences among the three eye groups. From controls, fellow eyes to the affected eyes, the amplitude decreased and the peak time shortened, with borderline P for trend ($P=0.053$ and 0.055, respectively). For PhNR2, however, no significant difference in either the amplitude ($P=0.259$) or the peak time ($P=0.083$) was found among the three eye groups. As shown in S Fig. 3 and S Table 1, the outcomes of the Bland–Altman analyses demonstrated good test–retest consistency between the right and left eyes, with a mean difference of less than 5% of the means. The Cronbach's alpha coefficients ranged from 0.76 to 0.81.

Correlation analyses

Figure 2 shows the relationship between PhNR1 and PhNR2 measurements, indicating a positive correlation between the two PhNR responses in both amplitude and peak time. The outcomes of the correlation coefficients between PhNR1 and PhNR2 are shown in Table 2. The four different correlation coefficients (PCC, ICC, CCC and KCC) were all significant in the whole sample as well as in each eye group (P values ranging from <0.001 to 0.006), with coefficient values ranging from 0.33 to 0.69. Most of the correlation coefficients were within the range of 0.4 ~ 0.6, indicating moderate strength of correlation.

The regression models

Based on GEE adjusting for correlation between paired eyes, the prediction models of PhNR1 with PhNR2 as a predictor were constructed in Table 3. Possible covariates were selected from age, sex, AL and IOP based on BICs. As shown in S Table 2, within a GEE framework adjusting for paired-eye correlation in the whole sample, the model with the lowest BIC was selected. Besides measurements of PhNR1 and PhNR2, no additional covariates were included in the final amplitude model, while age was added to the peak time model to improve predictive accuracy.

As shown in Table 3, the amplitude of PhNR1 cannot be accurately predicted by the amplitude of PhNR2 in any eye group or the whole sample. The RMSE was 4.72 μV with a MAPE of 36.7% in all the enrolled eyes. The percentage of eyes with an absolute percentage error (APE) within 25% of the PhNR1 amplitude was less than half (46.5%). On the other hand, predictions of the peak time were quite accurate, with an RMSE of 3.43 ms and MAPE of 3.9% in the whole sample. Additionally, the percentage of eyes with APE within 5%, 10% and 25% were 70.9%, 95.9% and 100% in the model that included all eyes.

Discussion

As far as we know, this is the first study focusing on the associations between two different protocols to obtain PhNR, providing important information for evaluating

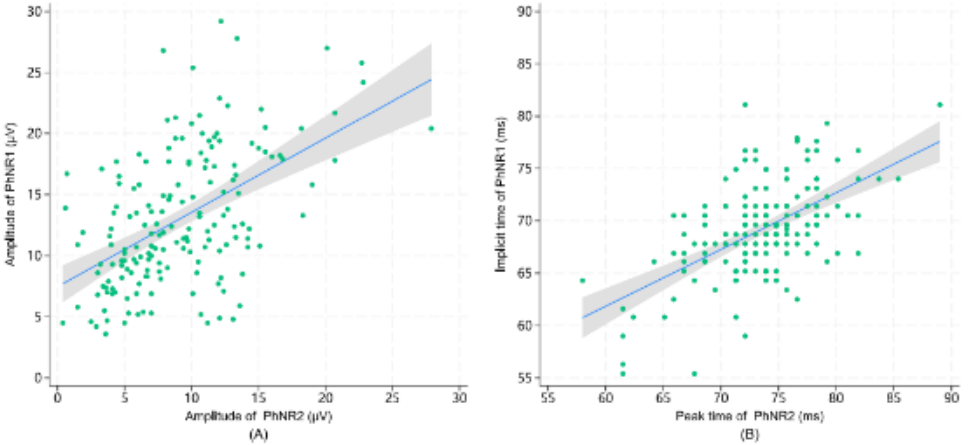

Fig. 2 The scatter plots and fitting lines between PhNR1 and PhNR2 for (**A**) amplitude (**B**) peak time. (PhNR1 = PhNR responses measured using the ISCEV extended protocol of PhNR, PhNR2 = PhNR responses measured using the ISCEV standard protocol for light-adapted 3.0 ERG)

Table 2 The correlation analyses between PhNR1 and PhNR2.*

	Eye group	PCC		ICC		CCC		KCC	
		Coefficient (95% CI)	*P*	Coefficient (95% CI)	*P* value	Coefficient (95% CI)	*P* value	Coefficient (95% CI)	*P* value
PhNR Amplitude, z-score	Controls	0.5 (0.25, 0.68)	< 0.001	0.43 (0.14, 0.65)	< 0.001	0.43 (0.22, 0.63)	< 0.001	0.37 (0.21, 0.53)	< 0.001
	Fellow eyes	0.69 (0.47, 0.83)	< 0.001	0.69 (0.47, 0.83)	< 0.001	0.69 (0.51, 0.86)	< 0.001	0.57 (0.42, 0.72)	< 0.001
	Affected eyes	0.52 (0.35, 0.66)	< 0.001	0.51 (0.33, 0.65)	< 0.001	0.50 (0.35, 0.65)	< 0.001	0.33 (0.21, 0.46)	< 0.001
	All	0.52 (0.4, 0.62)	< 0.001	0.52 (0.4, 0.62)	< 0.001	0.52 (0.41, 0.63)	< 0.001	0.36 (0.26, 0.45)	< 0.001
Peak time, z-score	Controls	0.62 (0.41, 0.77)	< 0.001	0.57 (0.31, 0.74)	< 0.001	0.57 (0.39, 0.74)	< 0.001	0.53 (0.33, 0.73)	< 0.001
	Fellow eyes	0.46 (0.14, 0.68)	0.006	0.42 (0.12, 0.66)	0.003	0.42 (0.16, 0.68)	0.002	0.37 (0.2, 0.54)	< 0.001
	Affected eyes	0.59 (0.43, 0.71)	< 0.001	0.59 (0.43, 0.71)	< 0.001	0.59 (0.45, 0.72)	< 0.001	0.34 (0.19, 0.48)	< 0.001
	All	0.57 (0.46, 0.66)	< 0.001	0.57 (0.46, 0.66)	< 0.001	0.57 (0.47, 0.67)	< 0.001	0.36 (0.27, 0.46)	< 0.001

PhNR1 = PhNR responses measured using the ISCEV extended protocol of PhNR, PhNR2 = PhNR responses measured using the ISCEV standard protocol for light-adapted 3.0 ERG

* *PCC* Pearson's correlation coefficient; *ICC* Intraclass correlation coefficient; *CCC* Lin's concordance correlation coefficient; *KCC* Kendall's consistency coefficient

studies of PhNR with different protocols and designing future studies on PhNR.

PhNR was first named by Viswanathan, S., et al. to indicate the negative-going response after the b-wave in LA ERG [2]. It was found to be reduced in macaque monkeys with experimental glaucoma [2], and similar findings were soon confirmed in humans with glaucoma damage [15].

In early studies of PhNR, the evoked conditions were broadband (white stimulus on white background), and many were based on the ISCEV protocol of light-adapted ("cone-response") ERG, first released in 1989 [16] and most recently updated in 2022 [3]. However, recent studies support narrow-band stimuli as more efficient in evoking a PhNR response [4, 5] and more sensitive in clinical diagnosis [17]. Consequently, ISCEV released the extended protocol for PhNR in 2018, characterized by using a narrowband stimulus (blue background and red light flash) during the exam [1]. This has led to heterogeneity in the studies on PhNR with different protocols. Therefore, this study is crucial for understanding different PhNR studies. Regarding PhNR definitions, previous studies have employed various approaches, including trough measurements before or after the i-wave [18], and fixed-time measurements like t = 65 ms [19] or 72 ms [20]. In our study, we adhered to the example figure in the ISCEV extended protocol for PhNR [1], measuring PhNR at the maximum trough following the initial i-wave. While the protocol permits a flexible time window of 65–75 ms post-flash, [1] we opted for the trough-based approach, as it is more widely adopted and less sensitive to timing variations associated with fixed-time measurements.

Our study indicates that analyses with PhNR evoked by different protocols may lead to different conclusions. In Table 1, both the amplitude and peak time of PhNR1 showed significant discrepancies among the three eye groups, with the P-for-trend at borderline significance (0.053 and 0.055). Based on PhNR1, both the amplitude and peak time presented a decreasing trend from controls, to fellow eyes and affected eyes, which may indicate the effect of amblyopia in pediatric cataract patients. In the published literature, we found two similar studies by Esposito V., et al., which reported similar attenuated amplitude of PhNR in both congenital and developmental cataract [9, 21], supporting our findings from PhNR1. On the other hand, based on PhNR2, no significant differences in neither amplitude or peak time were found among the three eye groups. While the available evidence, including previous studies [9, 21], suggests that PhNR1 may be more sensitive in detecting differences among eye groups, further research is necessary to definitively establish its superiority. The significance of the P-values found for PhNR1 may be due to chance, and the evidence supporting the constriction of PhNR in amblyopia remains limited. The PhNR/b-wave amplitude ratio appears to have a negligible impact on the inter-group comparisons in this study, as no significant variations were observed in either Ratio1 or Ratio2 across the three eye groups. The significantly larger Ratio1 compared to Ratio2 aligns with previous research demonstrating the superior efficacy of narrowband stimuli (e.g., blue background and red light flash in the ISCEV extended PhNR protocol) over broadband stimuli in LA 3.0 ERG [4, 5]. Given similar b-wave amplitudes, the more than twofold difference in medians (1.42 for PhNR1 and 0.64 for PhNR2) suggests that PhNR1 may provide a more efficient neural representation than PhNR2.

Both the amplitude and peak time showed a positive correlation between PhNR1 and PhNR2 (Fig. 2). Besides Pearson's correlation coefficient (PCC), which is a

Table 3 The predictive models for the amplitude and peak time of PhNR1 using PhNR2 as a predictor, adjusted for paired-eye correlation with the generalized estimating equation method*

		Mean PhNR1	Mean PhNR2	Fitting line	*P*	RMSE	MAPE	*n*% with APE		
								≤ 5%	≤ 10%	≤ 25%
Amplitude (μV)	Controls	15.2 (4.7)	8.8 (4.0)	A1 = 0.544 × A2 + 10.468	< 0.001	4.07	27.5%	12.5%	16.7%	54.2%
	Fellow eyes	13.1 (6.0)	9.4 (4.5)	A1 = 0.797 × A2 + 5.529	< 0.001	4.65	30.7%	12.7%	22.5%	50.7%
	Affected eyes	11.8 (5.4)	9.2 (5.2)	A1 = 0.320 × A2 + 7.801	0.015	4.08	41.1%	13.2%	18.9%	37.7%
	All	13.0 (5.5)	9.1 (4.7)	A1 = 0.548 × A2 + 7.853	< 0.001	4.72	36.7%	7.6%	15.7%	46.5%
Peak time (ms)	Controls	69.7 (3.6)	75.5 (3.2)	T1 = 0.609 × T2-0.386 × Age + 26.498	< 0.001	2.80	3.0%	83.3%	97.9%	100.0%
	Fellow eyes	69.6 (3.7)	72.5 (3.4)	T1 = 0.531 × T2-0.551 × Age + 33.94	< 0.001	3.24	3.9%	74.6%	95.8%	100.0%
	Affected eyes	68.7 (4.9)	72.8 (5.3)	T1 = 0.402 × T2-0.599 × Age + 43.384	< 0.001	4.04	4.6%	60.4%	90.6%	100.0%
	All	69.2 (4.4)	73.5 (4.6)	T1 = 0.440 × T2-0.566 × Age + 40.484	< 0.001	3.43	3.9%	70.9%	95.9%	100.0%

RMSE Root-mean-square error, *MAPE* Mean absolute percentage error, *APE* Absolute percentage error

PhNR1 = PhNR responses measured using the ISCEV extended protocol of PhNR, PhNR2 = PhNR responses measured using the ISCEV standard protocol for light-adapted 3.0 ERG

parameter-based measurement of linear fitness, three other commonly used correlation coefficients were also analyzed: ICC, CCC, and KCC, using the z-score transformed PhNR measurements. The ICC measures correlation based on the analysis of variance and treats the data in groups rather than matched pairs; the CCC reflects agreement by combining both the linear fitness and the deviation of the line from (x = y); the KCC, on the other hand, measures the correlation using a non-parameter based rank method [13, 14]. Different correlation coefficients were calculated in this study to enhance the robustness of the conclusions. Table 2 showed that the positive correlation is significant in each eye group as well as the whole sample for all four analyzed correlation coefficients (P ranges from < 0.001 to 0.006). We can conclude that both the amplitude and the peak time measured by the two protocols were significantly correlated with each other with very high certainty. On the other hand, in Table 2, most of the correlation coefficients were between 0.4 and 0.6, indicating a moderate correlation between measurements with the two protocols. This reveals that PhNR2 may reflect some information about PhNR1, but is insufficient as a replacement for the latter.

In Table 3, we evaluated the degree to which the measurements of PhNR1 could be linearly predicted by PhNR2. Since PhNR1 was more sensitive in diagnosis [9, 21], the models aimed to explore whether PhNR1 could be effectively predicted by PhNR2, which may be applied in studies with only PhNR2. Besides PhNR1 and PhNR2 measurements, we applied BIC in selecting the proper covariates from age, sex, IOP and AL to avoid overfitting and keep the model simple [22]. As shown in S Table 2, from the form of the model with the lowest BIC, we can see none of the covariates contributing much information in understanding the relationship between the amplitude of PhNR1 and PhNR2. For the peak time, on the other hand, the addition of age to the models improved prediction accuracy with a low possibility of overfitting, since the BIC decreased after introducing age in the model.

In Table 3, we can see the amplitude of PhNR1 cannot be accurately predicted by PhNR2 across all three eye groups. The MAPE is over one-third in the whole sample (36.3%), and less than half (46.5%) of the predictions with an APE ≤ 25%. These outcomes indicate the prediction model of the amplitude is seemingly impractical for use. The models for the peak time, however, are quite precise, with a MAPE of 3.9% in the whole sample and small errors across all eye groups. In the whole sample, over 95% of predictions have an APE ≤ 10%. Although external validity is not proved, the prediction model of the peak time appears to be quite efficient and practical. The different performance in the prediction models may be explained by the following reasons. Firstly, the variance of PhNR amplitude is much larger than the peak time in population, which increases the

difficulty of making accurate prediction. In this study, the coefficients of variance (CVs) for the amplitude were 0.42 (PhNR1) and 0.52 (PhNR2), while the CVs for the peak time were 0.06 (PhNR1) and 0.06 (PhNR2). The high variance of the amplitude has been proved by previous publications [23]. Secondly, the signal-to-noise ratio for the skin–electrode in this study was reported to be lower than the contact lens electrode [24], which may introduce measurement errors that reduce the performance of the models. Many studies on PhNR have only analyzed the amplitude [4, 25, 26]; however, if the peak time of PhNR can be associated with certain diseases or clinical characteristics, then it would be sufficient to only measure PhNR1 or PhNR2, as the other can be precisely predicted.

Limitations

We should note some limitations of this study in interpreting the findings. Firstly, pupil size was not measured, which may affect the amplitude values. However, the potential bias from small pupils is assumed to be minimal since pupil dilation was performed in all participants. Secondly, all patients and controls were recruited from the same hospital, which may introduce bias. Thirdly, all participants were children, and the findings may differ in adults, necessitating future studies with other population characteristics. Moreover, we used skin electrodes in children for comfort and better cooperation, which may introduce larger measurement errors compared to corneal electrodes [24].

Conclusions

The outcomes may differ with measurements of PhNR1 and PhNR2. PhNR1 appears to be a more sensitive measure than PhNR2 for detecting eye group differences. While significantly correlated, the moderate correlation coefficient and low prediction precision of amplitude suggest that PhNR2 may not be a complete substitute for PhNR1. The ISCEV specialized protocol may offer increased sensitivity, but further research is needed to definitively establish its superiority.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00417-024-06718-0.

Author contribution YEZ and BZ contributed to the design of the study. YEZ have full access to the data in the study. JW, YW, and YJ contributed to data collection, entry and management. BZ performed the data analyses. All authors contributed to data interpretation. BZ drafted the manuscript, all, authors critically revised it and approved the final manuscript.

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Data availability The data of this study are available at reasonable request.

Code availability The STATA code of this study are available upon request.

Declarations

Ethical approval and consent to participate This study was approved by the ethics committee of the Eye Hospital of Wenzhou Medical University (Reference No. 2020–089-K-81–01) and adhered to the tenets of the Declaration of Helsinki. All participants' parents or guardians provided informed consent. No animal subjects were included in this study.

Competing interests The authors declare no competing interests.

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