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ORIGINAL RESEARCH ARTICLE

Evaluation of light- and dark-adapted ERGs using a mydriasis-free, portable system: clinical classifications and normative data

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Abstract

Purpose The full-field electroretinogram (ff-ERG) is a widely used clinical tool to evaluate generalized retinal function by recording electrical potentials generated by the cells in the retina in response to flash stimuli and requires mydriasis. The purpose of this study was to determine the intra-visit reliability and diagnostic capability of a handheld, mydriasis-free ERG, RETeval (LKC Technologies, Gaithersburg, MD, USA), in comparison with the standard clinical ff-ERG by measuring responses recommended by the International Society for Clinical Electrophysiology of Vision (ISCEV).

Methods This prospective, cross-sectional study included 35 patients recruited at the Hospital for Sick Children (median age = 17, range 11 months–69 years) who had undergone a clinical ff-ERG according to ISCEV standards. For RETeval (n = 35), pupils were undilated in most (n = 29) and sensor strip electrodes were placed under the inferior orbital rim. Stimulus settings on RETeval were equivalent to those used in the clinical ERG. Fifty-seven control participants (median age = 22, range 8–65 years) underwent undilated RETeval ERG to establish standard values for comparison. Patient waveform components with amplitudes < 5th percentile, or implicit times > 95th percentile of normal relative to control data were classified as abnormal for the RETeval system.

Results The RETeval system demonstrated a high degree of within-visit reliability for amplitudes (ICC = 0.82) and moderate reliability for implicit times (ICC = 0.53). Cohen's Kappa analysis revealed

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a substantial level of agreement between the diagnostic capability of RETeval in comparison with clinical ff-ERG (k = 0.82), with a sensitivity and specificity of 1.00 and 0.82, respectively. Pearson's correlations for clinical ERG versus RETeval demonstrated a positive correlation for amplitudes across the rod (r = 0.65) and cone (r = 0.74) ERG waveforms. Bland–Altman plots showed no bias between the mean differences across all amplitude and implicit time parameters of the two systems.

Conclusions The present study demonstrated that RETeval is a reliable tool with reasonable accuracy in comparison with the clinical ERG. The portable nature of RETeval system enables its incorporation at resource-limited centers where the ff-ERG is not readily available. The avoidance of sedation and pupillary dilation are added advantages of RETeval ERG.

Keywords Electroretinography · RETeval · Handheld electroretinography · Mydriasis-free

Introduction

The full-field electroretinogram (ff-ERG) is used in clinical centers worldwide to assess generalized retinal function. The benchmark for ERG testing is published by the International Society for Clinical Electrophysiology of Vision and specifies six sets of recording protocols based on the adaptation state of the eye and stimulus strength [1]. These include (1) Dark-adapted 0.01 ERG (rod ERG); (2) Dark-adapted 3.0 ERG (combined rod-cone standard flash ERG); (3) Dark-adapted 3.0 oscillatory potentials; (4) Dark-adapted 10.0 ERG (strong flash ERG); (5) Light-adapted 3.0 ERG (standard flash "cone" ERG); and (6) Light-adapted 30-Hz flicker ERG. The standard protocols require that the participants' pupils be dilated to standardize the amount of light reaching the retina and the placement of electrodes that contact the corneal surface to maximize signal to noise ratio [2]. However, conventional ERG recordings are not easily obtained awake in young children and hence may require sedation or anesthesia especially in those with developmental disability. Additionally, ff-ERG may not be available in hospitals/clinical environments where resources are limited.

RETeval (LKC Technologies, Gaithersburg, MD, USA) is a system for recording ERGs that consists of a handheld stimulator and signal recorder coupled with custom, disposable, electrodes [3]. RETeval eliminates the need for mydriatics and compensates for changes in the pupillary area (mm^2) by adjusting the flash luminance ($cd\text{-}s/m^2$) to maintain a constant retinal illuminance (Td-s) and is stable for pupil diameters of up to 6.5 mm [3, 4]. It is designed to use custom sensor strips that adhere to the skin below the lower eyelid; this is expected to allow a higher tolerance of testing in children who may prefer skin electrodes as opposed to the corneal/conjunctival contact electrodes used by conventional ERG. Bradshaw et al. [5] compared ERG waveforms recorded with skin and corneal electrodes in healthy children and adults; the recorded amplitudes are lower as expected with skin electrodes, while the waveform morphology of dark- and light-adapted ERGs was comparable to corneal electrodes. After scaling the skin electrode ERG distribution by 4.5 times (Range 2.6 times for light-adapted 3.0 ERG *b*-wave—5.4 times for light-adapted 3.0 ERG *a*-wave), the amplitudes were comparable across all stimulus parameters. The effectiveness of the RETeval system has been evaluated in several studies including screening for diabetic retinopathy (DR) and retinal degeneration [6–8]. Much of the literature concentrates primarily on signals recorded using the light-adapted 30-Hz flicker protocol, which does not address dark-adapted function. The purpose of this prospective cross-sectional study was to determine reliability and accuracy of the handheld, mydriasis-free ERG, RETeval, in comparison with the standard clinical ff-ERG.

Methods

Participants

Thirty-five patients referred (median age = 17, range 11 months–69 years) to the Visual Electrophysiology Unit (VEU) at the Hospital for Sick Children (SickKids) for ophthalmological, visual, and electrophysiological examination were recruited. The spectrum of causative diseases, consisting of both inherited and acquired retinal conditions, is listed in Table 1. Patients with photosensitive epilepsy, structural damage to the bony orbit, and infectious diseases of the eye

(i.e., conjunctivitis and uveitis) were excluded from the study. All patients recruited underwent psychophysical tests consisting of visual acuity, color vision, and contrast sensitivity. The ISCEV standard Ganzfeld ff-ERG testing [1] was completed in all except 3 patients (n = 32). Of the three patients, one 11-month-old patient (ID 678), with a diagnosis of horizontal conjugate nystagmus and iris coloboma, did not undergo clinical ERG as the family refused sedated examination. This child's RETeval ERG was normal, and the retinal examination was normal. Patients ID 628 with myopic astigmatism and ID 669 with subnormal visual acuity did not have available ff-ERG measurements but they underwent pattern ERG and multi-focal ERG, respectively. Data from these three patients were only included in the calculation of RETeval intra-visit reliability.

For the 32 patients who had both handheld and clinical ERG assessments, RETeval ERG was conducted on a separate visit in 26 participants, the 6 remaining patients consented for participation on the same day (between 1 and 6 h post-dilation).

Fifty-seven control participants (median age = 22, range 8–65 years) with no known retinal disease were recruited from the general population and underwent RETeval ERG. These included SickKids members of staff and their families, students and families of patients; all of whom had normal best corrected visual acuity (VA; ETDRS chart), contrast sensitivity (Smart System II PC-Plus, M&S Technologies, Inc., Niles, Illinois), color vision (Mollon-Reffin minimal color test), and no known retinal disorder. Forty-seven of the control participants also underwent ophthalmoscopic evaluation by a registered optometrist at SickKids. Nine control participants had a normal eye examination with an optometrist/ophthalmologist outside of SickKids Hospital whereas one was self-reported normal having no prior subjective experiences with visual deficits.

The protocol was approved by the Research Ethics Board at the Hospital for Sick Children and conformed to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all patients, control participants, and parents (when participants were younger than 16 years of age) after the details of the procedures as well as questions pertaining to risks and harms of the study were fully addressed.

Ganzfeld full-field electroretinography

The patient's eyes were dilated using 1% tropicamide (Mydriacyl; Alcon Laboratories Inc, Texas, USA) and 2.5% phenylephrine (Mydfrin; Alcon Laboratories Inc, Texas, USA). Participants then dark-adapted by sitting in a dark room wearing additional eye patches for 20 min. After dark adaptation and administration of a topical anesthetic, DTL fiber electrodes (Diagnosys LLC, Lowell, MA, USA) were placed in front of the eyes [9]. The ff-ERG was conducted using Ganzfeld stimulator (Espion E2 Color Dome, Diagnosys LLC). The dark-adapted 0.01 ERG (rod ERG), dark-adapted 3.0 ERG (combined rod-cone standard flash ERG), and dark-adapted 10.0 ERG (strong flash ERG) were recorded [1]. Following recording of dark-adapted ERGs, participants were light adapted (at 30 cd/m^2) for 10 min. After light adaptation, a second series of flash stimuli were used to test for single-flash cone ERGs (light-adapted 3.0 ERG 2 Hz) and 30-Hz flicker. The intensity of the stimuli and the background light were specified in accordance with the published protocols incorporating ISCEV standard for clinical electroretinography [1]. All steps of the clinical ff-ERG were repeated two to three times to ensure intra-test repeatability, and two of the repeated trials are represented in Figs. 3 and 4.

RETeval electroretinography

For RETeval, the background and flash luminance were equivalent for the ISCEV standard stimuli assuming an 8 mm pupil is achieved with pharmacodilation. Both patients and controls dark-adapted for 20 min in a light-free room wearing an opaque blindfold on top of eye patches. The disposable electrodes (sensor strips) were placed at 2 mm below the lateral half of the lower lid margin, after the skin was cleaned with a mild dermabrasive gel (Nuprep®). Following dark adaptation, the RETeval device delivered dim stimuli via intermittent flashes with strengths of 0.28 Td.s (equivalent to 0.01 cd.s/m^2), 85 Td.s (equivalent to 3 cd.s/m^2), and 280 Td.s (equivalent to 10 cd.s/m^2) in a small Ganzfeld dome of 60 mm in diameter. Eye position stability was maintained by encouraging fixation on a red central fixation dot inside of the stimulator. Fixation was monitored by viewing the pupil through the RETeval device. Trials were performed in duplicates to assess within-visit

Table 1 Demographics data and ERG classification results for patients referred to the VEU

ID	Sex	Age at testing (years)	Visual acuity (OD)	Visual acuity (OS)	Clinical diagnosis	Clinical ERG classification	RETeval classification
602	M	9	20/125	20/160	Congenital nystagmus	Normal (borderline)	Normal rod function (cone function not measured)
603	M	30	20/20	20/20	Benign fleck retina	Normal	Normal
622	M	14	20/25	20/25	Retinitis pigmentosa	Generalized rod and cone dysfunction	Generalized rod and cone dysfunction
623	M	69	20/20	20/20	Suspected acute zonal occult outer retinopathy	Generalized rod and cone dysfunction	Generalized rod and cone dysfunction
624	M	11	20/50	20/50	Spina bifida, Nyctalopia	Normal	Normal
627	F	32	20/16	20/16	Branch retinal arterial occlusion	Normal	Normal
628	F	6	20/32	20/32	Myopic astigmatism	Normal pattern ERG	Generalized cone dysfunction
629	M	27	20/32	20/32	Night blindness, normal fundus	Normal	Normal
631	M	52	20/25	20/25	Ocular hypertension	Normal	Mild generalized rod and cone dysfunction
638	F	46	N/A	N/A	Dominant drusen	Normal	Normal
641	M	9	20/40	20/40	Congenital stationary night blindness	Rod inner retinal dysfunction and generalized cone dysfunction	Rod inner retinal dysfunction and generalized cone dysfunction
645	M	9	20/20	20/25	Myopic astigmatism	Normal	Normal
647	M	59	20/125	20/32	Bilateral optic neuropathy	Normal	Normal
653	F	11	20/32	20/40	Hurler Syndrome	Selective inner retinal dysfunction of rod system	Generalized rod and cone dysfunction
657	M	16	20/80	20/63	Optic neuropathy	Mild inner retinal dysfunction affecting the rod system, borderline cone response	Rod inner retinal dysfunction and generalized cone dysfunction
659	F	57	20/20	20/20	Stargardt's macular dystrophy	Normal	Mild generalized cone dysfunction
660	M	21	20/50	20/40	Vitelliform macular dystrophy	Normal	Normal
661	M	18	0.9 logmar	0.84 logmar	ABCA4 retinopathy	Generalized cone dysfunction	Generalized cone dysfunction
663	F	5	N/A	N/A	Foveal hypoplasia	Normal	Generalized cone dysfunction (rod functions not measured)
664	M	66	20/100	20/125	Congenital stationary night blindness	Rod inner retinal dysfunction and generalized cone dysfunction	Rod inner retinal dysfunction and generalized cone dysfunction

Table 1 continued

ID	Sex	Age at testing (years)	Visual acuity (OD)	Visual acuity (OS)	Clinical diagnosis	Clinical ERG classification	RETeval classification
665	M	36	20/200	20/100	Bilateral maculopathy	Normal	Normal
668	M	11	20/32	20/40	Optic neuropathy	Normal	Normal rods, borderline cone function
669	M	8	20/50	20/32	Bilateral maculopathy	Abnormal pattern ERG	Normal cone function (rod function not measured)
670	M	15	20/250	20/250	ABCA4 retinopathy	Generalized cone and rod dysfunction	Generalized rod and cone dysfunction
671	M	49	20/50	20/25	Asymmetric retinopathy	Normal (left eye)	Normal cone function (rod function not measured) left eye
672	M	17	LP	Prosthetic eye	Bilateral retinoblastoma	Generalized rod and cone dysfunction	Generalized rod and cone dysfunction
673	M	21	20/50	20/100	Kearns-Sayre syndrome	Generalized rod and cone dysfunction	Generalized rod and cone dysfunction
674	F	27	20/160	20/125	Retinitis pigmentosa	Generalized rod and cone dysfunction	Generalized rod and cone dysfunction
678	F	11 m	F + F	F + F	Horizontal conjugate nystagmus, Coloboma	Not available	Normal
679	M	11	20/40	20/50	Optic nerve dysfunction	Normal	Normal
683	F	15	20/80	20/125	Bilateral maculopathy	Normal	Normal
684	F	63	20/40	20/32	Bilateral maculopathy	Borderline generalized rod and cone responses	Generalized rod and cone dysfunction
685	F	15	20/25	20/32	Retinitis pigmentosa	Generalized rod and cone dysfunction	Generalized rod and cone dysfunction
688	M	21	20/40	20/40	Congenital stationary night blindness	Rod inner retinal dysfunction and generalized cone dysfunction	Rod inner retinal dysfunction and generalized cones dysfunction
689	M	37	20/12	20/25	Autoimmune retinopathy	Generalized rod and cone dysfunction	Generalized rod and cone dysfunction

Note participant 678 was unable to have recordable clinical ERG because the parents refused sedation and results were deemed normal by VEU medical director (AV). Patients 628 and 669 did not have available ff-ERG and underwent pattern ERG, their responses were subsequently determined as normal or abnormal based on the results of the pattern ERG and unremarkable clinical examinations. Patient 671 and 669 declined scotopic testing for RETeval. Patient 663 could not complete scotopic testing due to horizontal nystagmus. Patient 602 declined photopic testing due to discomfort. *LP* light perception, *F + F* fixation and following

reliability of each stimulus condition after verifying recorded ERG waveforms from previous trial and repositioning the mini-Ganzfeld stimulator. Participants then performed light adaptation at a background luminance of 30 cd/m^2 (848 Td) for 10 min with Ganzfeld held in front of the tested eye before resuming light-adapted testing using 85 Td.s (3 cd.s/m^2 2 Hz) and 30-Hz flicker (85 Td.s or 3 cd.s/m^2). Both eyes were tested sequentially (right eye followed by the left eye) in each participant. Table 2 shows the

stimulus parameters and testing sequence for the RETeval device. Each protocol was repeated immediately in succession to assess for intra-visit reliability. Data from both eyes were included independently for the secondary analysis of measures of agreement but the data and analysis subsequently displayed are the right eyes for controls and the eye with the better waveform for patients as evidenced by clinical ERG waveforms.

In the youngest tested subject (ID 678, 11 months old), RETeval was performed awake with the infant wrapped within a blanket and with the parents remaining in close proximity. The pupil viewed through the RETeval device allowed assessment of stability of eye movements. Eyelid speculum was not required as the child remained calm and there was no excessive crying.

Pupils were undilated during RETeval ERG in most ($n = 29$) patients. The remaining 6 patients had undergone a dilated clinical full-field ERG and fundus examination prior to RETeval testing on the same day and it was inconvenient for them to return for a second visit due to distance and scheduling constraints.

Data analysis

Three age groups were established for data analysis ($\leq$ 20 years; 21–40 years; and $\geq$ 41 years) based on the study by Nakamura et al. [8] which found significant amplitude and implicit time differences between patients with retinal dysfunction and controls in each of these three age groups using RETeval.

Reliability was assessed by comparing two separate ERG waveforms within a visit (intra-visit reliability), and intra-class correlation coefficient (ICC) was calculated to determine the repeatability of the RETeval amplitudes and implicit times. Accuracy was determined by comparing diagnostic accuracy of the RETeval with the standard clinical ERG. For assessment of accuracy, age-matched normative data exists for the standard clinical ERG while control data for RETeval were collected as part of this study ($n = 57$) to establish limits of normality for the handheld ERG. For the accuracy analysis, the first trial of the right eye was chosen for control participants on the RETeval ERG. For the patients, the eye with the better waveform was chosen on the clinical and RETeval ERGs. ERG results with amplitudes $<$ 5th percentile, or implicit times $>$ 95th percentile of normal were considered abnormal. Non-recordable ERG waveforms were designated with an amplitude of 0 and their implicit times were excluded from further analysis. A 2 $\times$ 2 contingency table was used to demonstrate the numbers with normal versus abnormal waveforms for both standard ERG and RETeval assessment. Assessing the diagnostic accuracy of the RETeval was the primary question of this study.

To determine the level of agreement between RETeval and standard ERG, Cohen's Kappa Statistic

Table 2 RETeval stimulus presentation sequence

Step	Description	Eye	Flash luminance energy (Td.s)	Flash luminance energy (cd.s/m^2)	Background luminance	# Flashes
1	Dark adaptation timer	Both	Off	Off	Off	
2	Dark-adapted 0.01 ERG	Right	0.28	0.01	Off	9 @ 0.5 Hz
3	Dark-adapted 3.0 ERG	Right	85	3	Off	5 @ 0.1 Hz
4	Dark-adapted 10.0 ERG	Right	280	10	Off	5 @ 0.05 Hz
Steps 2–4 are performed in the left eye						
5	Light adaptation timer	Right	Off	Off	30 cd/m^2 (848 Td)	
6	Light-adapted 3.0 ERG	Right	85	3	30 cd/m^2 (848 Td)	30 @ 2 Hz
7	Light-adapted 30-HZ flicker ERG	Right	85	3	30 cd/m^2 (848 Td)	141–424 @ 28.3 Hz
Steps 2–4 are performed in the left eye						

Note flash illuminance energy obtained at 8 mm pupil size

was used with a value of kappa higher than 0.75 demonstrating excellent agreement while lower than 0.4 indicating poor agreement. For patients that had abnormal ERGs, we also subclassified the patient according to their type of retinal defect across the two modalities for direct comparison.

Secondary analysis was performed to determine the association between standard ERG and RETeval ERG waveforms. Descriptive statistics using Pearson's correlation were used to establish the degree of association between RETeval and clinical ERGs across dark-adapted and light-adapted conditions. Analysis was carried out using Microsoft Excel spreadsheet (Microsoft, Corp., Redmond, WA) and Statistical Package for Social Studies (SPSS) (IBM, Chicago, IL).

Additionally, the Bland–Altman test was conducted to evaluate the presence of bias between the mean difference of RETeval and clinical ERG results [10]. For this analysis, amplitudes were scaled using a scaling factor of 6.62, i.e., clinical ERG amplitude = RETeval amplitude × 6.62. The Bland and Altman plot gives an estimate of agreement interval within which 95% of the differences of RETeval compared with clinical ERG lie. We expected a large interval as the skin and DTL electrodes are different in terms of amplitude of response and the scaling acts to increase noise in the skin electrodes. Although the Bland–Altman plot defines the intervals of agreements, it is not used to specify whether those limits are acceptable or not [10].

Results

Reliability

The intra-class correlation coefficient (ICC) for the RETeval device was computed for all participants included in the study (patients and controls, $N = 92$). Average implicit time ICCs across dark- and light-adapted ERG is moderate at 0.52 (range 0.28–0.99), whereas average ICCs for amplitudes is strong at 0.79 (range 0.58–0.92) (Refer to Table 3 for details).

Accuracy

Pearson's correlations for clinical ff-ERG versus RETeval demonstrated a positive correlation for amplitudes 0.62 for the DA 0.01b, 0.52 for DA 3.0a, 0.75 for DA 3.0b, 0.24 for LA 3.0a, 0.69 for LA 3.0b, and 0.74 for the LA 30-Hz flicker (Fig. 1). A moderate to strong positive correlation was also observed for implicit times 0.59 for the DA 0.01b, 0.77 for DA 3.0a, 0.59 for DA 3.0b, 0.31 for LA 3.0a, 0.66 for LA 3.0b, and 0.94 for LA 30-Hz flicker (Fig. 2). Data points for patients who underwent mydriasis are highlighted in red in Figs. 1 and 2; these data are consistent with data from the patients who did not undergo mydriasis.

Ranges for the right eye of control participants were computed to establish normative ranges for implicit time and amplitudes (see Table 4). Cohen's Kappa analysis revealed a substantial level of agreement between the diagnostic capability of RETeval in comparison with clinical ff-ERG ($k = 0.82$), with an overall sensitivity and specificity of 1.00 and 0.82, respectively. Each patient's retinal defect was subsequently classified into dysfunction involving the rods, cones, rod and cone or inner retinal according to the age-matched normative values for RETeval and clinical ERG. The ability to classify patients into subtypes of retinal defects is comparable between RETeval and ff-ERG as presented in Table 1. Within the cohort of patients ($n = 32$) who have completed assessments on both devices, 28/32 individuals (87.5%) showed matching electrophysiological diagnosis between the standard and RETeval ERGs.

Among the remaining patients ($n = 4$), two types of cross-modality classification discrepancy exist, 1) false-positive results on RETeval ($n = 3$), and 2) mismatch between inner retinal and generalized dysfunctions ($n = 1$). Among the three false positives, patient ID 631 (ocular hypertension) showed mild generalized rod-cone dysfunction, whereas IDs 659 (Stargardt's macular dystrophy) and 663 (foveal hypoplasia) showed mild cone dysfunction (see Table 1 for details). One patient (ID 653; Hurler Syndrome) with selective inner retinal rod dysfunction on the clinical ERG showed generalized rod and cone dysfunction on the RETeval ERG.

Representative ERGs are juxtaposed for the RETeval system and clinical ff-ERG in two patients (Figs. 3, 4; ID 622 and ID 629). Both dark-adapted and light-adapted ERGs were consistently attenuated across both modalities in the patient in Fig. 3 with a clinical diagnosis of retinitis pigmentosa. Figure 4 represents ERG traces from a patient with suspected

Table 3 Intra-visit reliability of RETeval ERG as shown by intra-class correlation coefficient (ICC) for all dark-adapted and light-adapted parameters

ICC values for both implicit time (ms) and amplitude (μV) are shown

ERG parameter	ICC—Implicit time (ms)	ICC—Amplitude (μV)
DA 0.01b	0.66	0.71
DA 3.0a	0.36	0.77
DA 3.0b	0.48	0.90
DA 10.0a	0.45	0.83
DA 10.0b	0.35	0.89
LA 3.0a	0.28	0.58
LA 3.0b	0.57	0.87
LA 30-Hz Flicker	0.99	0.92

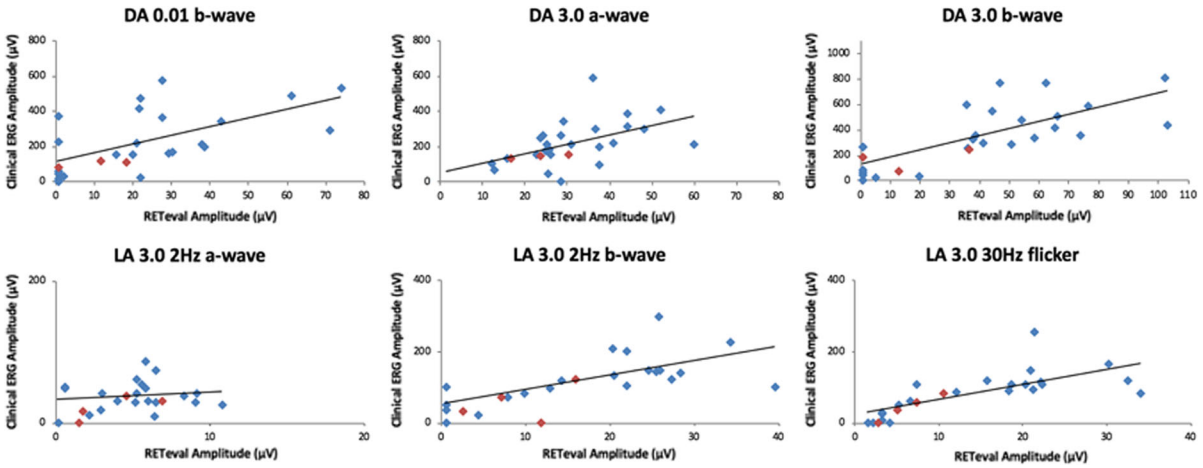

Fig. 1 Pearson's correlation for amplitudes of clinical ff-ERG versus RETeval demonstrate a moderately strong positive correlation across the stimuli tested **a** Dark-adapted (DA) 0.01 *b*-wave, **b** DA 3.0 *a*-wave, **c** DA 3.0 *b*-wave, **d** Light-adapted (LA) 3.0 2-Hz *a*-wave, **e** LA 3.0 2-Hz *b*-wave, and **f** LA 3.0 30-Hz flicker. Amplitudes that were below the threshold for recording were designated as 0.5 μV by default for RETeval and clinical ERG. Data points for patients who underwent mydriasis are highlighted in red

nyctalopia but had normal fundus examination; the ERGs were normal across both modalities.

Bland–Altman plots show no bias between the mean differences of both ERG systems in terms of amplitudes and implicit times (see Fig. 5 which uses the DA 3.0 *b*-wave amplitude and DA 3.0 *b*-wave implicit time as an example).

Completion rate

Within the patient group (n = 35), 18 participants (51.42%) were able to complete all five steps (DA 0.01, 3.0 and 10.0 ERGs & LA 3.0 and 30-Hz flicker ERGs) of RETeval ERG in either one or both eyes (Age range 11–69 years). Among the remaining seventeen (Age range 11 months–66 years), 13 were able to complete at least one of the three dark-adapted steps in addition to at least one of the two light-adapted testing in one or both eyes; three patients declined or did not complete dark-adapted assessments, and one individual declined light-adapted testing.

Discussion

This study establishes an age-specific normative range (8–65 years) for both dark-adapted and light-adapted ERGs based on the RETeval device and further validates the feasibility of the RETeval portable ERG as a reasonably effective tool for stratifying patients with suspected retinal conditions. The present study also demonstrated high within-session reliability for amplitudes of RETeval waveforms.

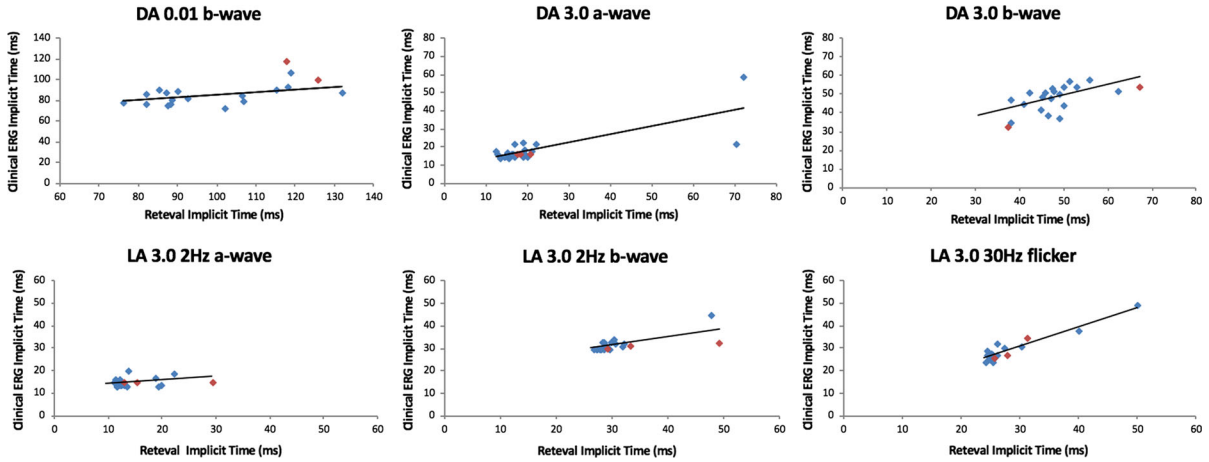

Fig. 2 Pearson's correlation for implicit times of clinical ff-ERG versus RETeval demonstrate a moderate to strong correlation across the stimuli tested **a** Dark-adapted (DA) 0.01 *b*-wave, **b** DA 3.0 *a*-wave, **c** DA 3.0 *b*-wave, **d** Light-adapted (LA) 3.0 2-Hz *a*-wave, **e** LA 3.0 2-Hz *b*-wave, and **f** LA 3.0 30-Hz flicker. Data points for patients who underwent mydriasis are highlighted in red

Normative ranges

In the existing literature, normative values for RETeval are limited. In a recent study by Asakawa et al. [11], normative ERG values using RETeval were reported in 50 healthy participants; however, this was across a narrow age range (20–24 years); this would not be representative because existing literature using conventional ERG (Ganzfeld) shows that b-wave amplitudes decrease with advancing age [12–15]. Further, the ff-ERG waveform from normal full-term infants resembles the adult ERG albeit with attenuated amplitudes for the first few months of life; also, the peak amplitude is typically achieved in adolescence [14] and there is evidence of slow decline from the sixth decade of life [13]. Furthermore, prolongation of implicit times is typically noted with aging [12]. In the present study, we established the normative values for the RETeval ERG in control participants across a wide age range (aged 8–65 years), and this would be more representative of age-matched normative data for all ERG parameters. We established these normative data to compare with the ERGs of patients across various age groups. The values obtained from the present study were comparable to Asakawa on average, but we did observe age-related variations in our data ranges, which led us to subsequently employ age-adjusted ranges to classify RETeval ERGs as normal versus abnormal.

Reliability

The intra-visit reliability established in present study was mostly comparable to previous reports using the RETeval device with ICCs averaging 0.53 for implicit times and 0.82 for amplitudes [11]. Asakawa et al. [11] demonstrated reasonable consistency for single session RETeval measurements with ICCs ranging from 0.48 to 0.91 for implicit time and ICCs ranging from 0.71 to 0.92 for amplitude. The slightly reduced ICC values to some parameters (LA 3.0 *a*-wave) observed in the present study may be contributed by a limited range of data point distribution for the LA 3.0 testing along with reduced amplitude in several of the waveforms that subsequently yielded inconsistent implicit time measurements.

Accuracy

Our study supports the finding that RETeval has a reasonable diagnostic accuracy in comparison with the clinical ff-ERG while exhibiting a high degree of sensitivity (1.00) and specificity (0.82). The screening potential of RETeval has been previously examined in a study by Maa et al. [6], demonstrating in 468 patients with vision-threatening diabetic retinopathy that the RETeval 30-Hz flicker exhibited a sensitivity and specificity of 0.83 and 0.78, respectively. Al-Otaibi et al. [16] further substantiated these findings by reporting a high degree of sensitivity of 95.4% with

Table 4 Percentile rank for RETeval by age in control participants

Age	Stimulus parameter	Implicit time percentile (ms)			Amplitude percentile (μV)		
		Median	5th	95th	Median	5th	95th
< 20 years (n = 25)	DA 0.01 rod—*b*-wave	83	68	100	41	26	69
	DA 3.0 rod/cone—*a*-wave	15	13	17	39	22	66
	DA 3.0 rod/cone—*b*-wave	43	34	48	56	42	98
	DA 10.0 rod/cone—*a*-wave	12	10	13	45	33	73
	DA 10.0 rod/cone—*b*-wave	47	33	49	62	50	100
	LA 3.0 cone—*a*-wave	11	10	13	6	4	9
	LA 3.0 cone—*b*-wave	28	27	30	27	17	36
	LA flicker cone	25	24	25	31	16	38
20-40 years (n = 20)	DA 0.01 rod—*b*-wave	86	71	96	36	19	63
	DA 3.0 rod/cone—*a*-wave	15	12	19	39	21	56
	DA 3.0 rod/cone—*b*-wave	49	40	59	71	35	104
	DA 10.0 rod/cone—*a*-wave	12	10	13	45	24	69
	DA 10.0 rod/cone—*b*-wave	50	41	62	69	36	105
	LA 3.0 cone—*a*-wave	12	9	14	6	0	11
	LA 3.0 cone—*b*-wave	29	27	30	22	16	38
	LA flicker cone	25	25	26	22	10	40
40-70 years (n = 12)	DA 0.01 rod—*b*-wave	83	68	103	41	21	79
	DA 3.0 rod/cone—*a*-wave	15	13	18	39	22	62
	DA 3.0 rod/cone—*b*-wave	44	35	52	61	42	86
	DA 10.0 rod/cone—*a*-wave	11	10	13	44	31	77
	DA 10.0 rod/cone—*b*-wave	47	35	53	64	54	95
	LA 3.0 cone—*a*-wave	11	10	13	6	4	9
	LA 3.0 cone—*b*-wave	28	26	30	26	17	38
	LA flicker cone	25	24	25	29	16	37

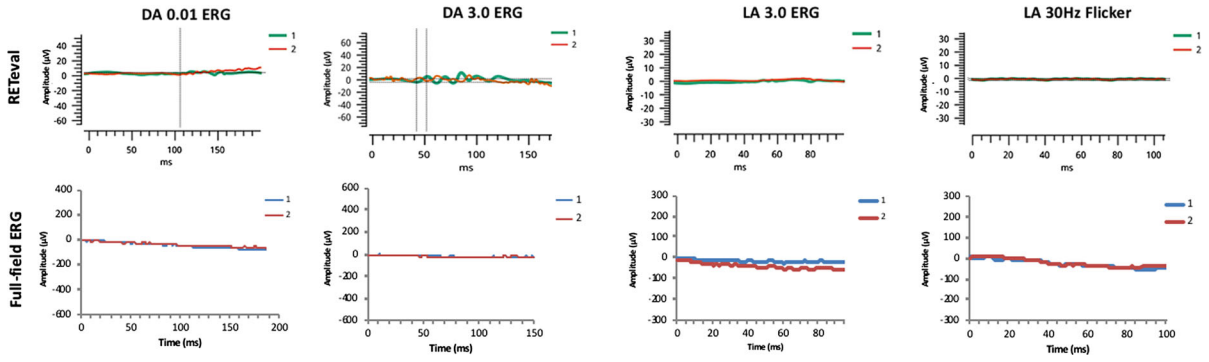

Fig. 3 Representative waveforms for a 14-year-old boy with retinitis pigmentosa for the RETeval and ff-ERG across both dark-adapted and light-adapted conditions. Note the horizontal axis starts at − 20 ms for RETeval recordings. The two trials are represented by separate colors. Both RETeval and Ganzfeld ERGs are non-detectable to all tested stimuli

the 30-Hz flicker in 400 patients with diabetes, supporting its applicability as a screening device clinically. Nevertheless, a comparison of diagnostic capability for both dark-adapted and light-adapted ERGs has not been examined thus far in patients with retinal defects. Our study corroborates the existing

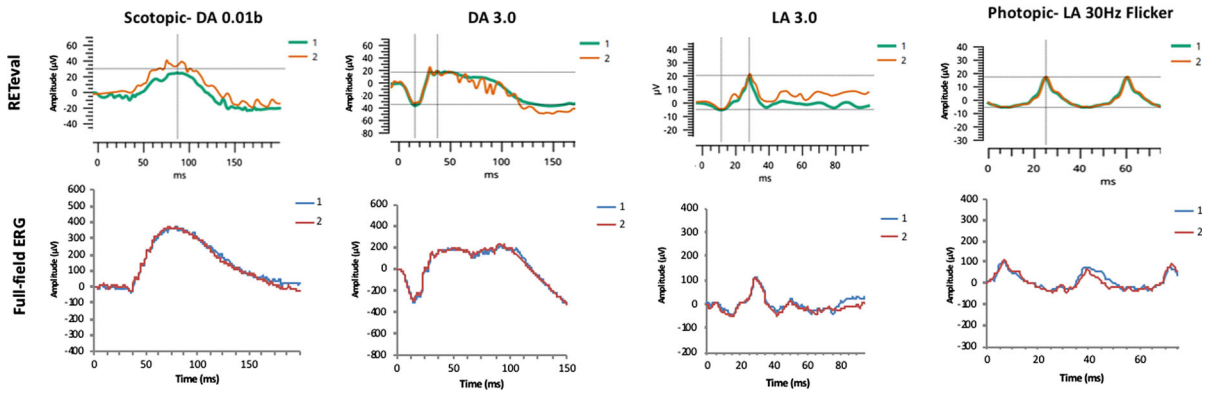

Fig. 4 Representative waveforms (RETeval and ff-ERG) for a 27-year-old man across both dark-adapted and light-adapted conditions. The patient had complaints of nyctalopia but fundus examination was normal. Note the horizontal axis starts at − 20 ms for RETeval recordings. The two trials are represented by separate colors. Both RETeval and Ganzfeld ERGs are normal across all tested stimuli

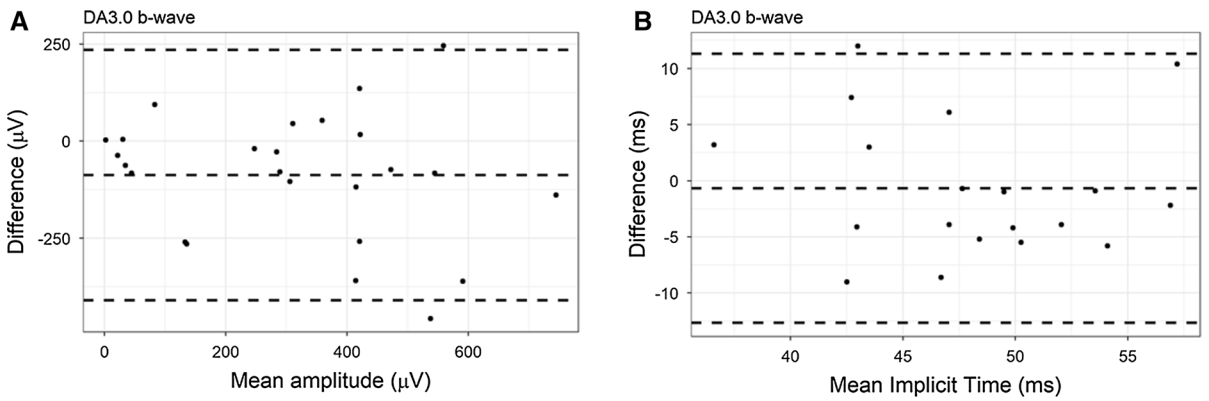

Fig. 5 Representative Bland and Altman plots for dark-adapted 3.0 *b*-wave amplitude (**a**) and implicit time (**b**) measures. The RETeval amplitudes were scaled to the clinical ff-ERG using a scaling factor of 6.62. No bias was observed between the mean differences of both ERG systems in terms of amplitudes and implicit times

literature by validating the feasibility of the RETeval portable ERG as an effective tool for stratifying patients with suspected retinal conditions given that 88% (28/32) of patients showed matching diagnosis as either normal or as specific retinal dysfunctions across both devices. In addition, all except for one patient determined as abnormal by both measuring modalities were correctly diagnosed with respect to the type of retinal defect (rod, cone, rod and cone or inner retinal). The consistency as well as the positive correlation with the clinical ERG demonstrated across both dark-adapted and light-adapted recording conditions suggests that the RETeval can be used in situations where clinical ERG is not feasible. Such conditions may involve children with behavioral and developmental concerns, or patients opting for more non-invasive diagnostic testing.

Causes for discrepancy

Among the seventeen patients who demonstrated normal waveforms on the clinical ERG, three (ID 631, ID 659, and ID 663) showed false-positive results on the RETeval ERG as mild generalized cone ± rod dysfunction. Five patients were identified with having inner retinal dysfunctions on the clinical ERG, and of these, four participants showed matching findings on the RETeval ERG, and one participant (ID 653) showed generalized rod and cone deficits on the RETeval ERG.

The false-positive results and the mismatch between generalized and inner retinal dysfunctions may be secondary due to excessive movement artifact of the eye in combination with undilated pupil which resulted in difficulties capturing reliable recordings on the RETeval system. The lower signal to noise ratio due to the use of RETeval sensor strip electrodes which produces lower amplitude waveforms at approximately 42% of those obtained with corneal electrodes may also contribute to discrepancy [17]. The skin electrodes were found to be less sensitive than the corneal counterparts based on previous linear regression analysis by Leguire et al. [18]. It is also to note that under non-mydriasis testing conditions, the RETeval Ganzfeld would illuminate the central retina up to approximately 120° for all pupil diameters; however, it is unknown whether regions outside of this field are equally stimulated.

Limitations

Firstly, a relatively confined sample size and age range due to recruitment specificities may restrict the generalizability of the results for individuals with other retinal conditions not encompassed in this study. Secondly, the study population was small and participants varied in terms of ethnicity, age, sex, and iris color (majority had brown iris colors), which would affect the power of the findings. Individual variations such as pupil size and iris pigmentation could affect RETeval results, as the device requires constant pupil detection to provide adequate illuminant stimulus [19]. As noted, $\sim$ 49% patients (17/35) could not complete all steps of the RETeval ERG protocol. In the present study, six out of 35 participants underwent pupillary dilation prior to RETeval assessment which may affect the interpretation of the implicit time measures in these selected patients [2]. Finally, the ERG waveforms are known to be electrode specific [1], however, our current study was not designed to compare the effectiveness of the RETeval skin electrodes to the DTL electrodes used by the clinical ERG.

Conclusion

To the best of our knowledge, this study was the first in the literature to establish the age-specific normative ranges of both dark-adapted and light-adapted ERGs based on the RETeval technique. The portable ERG possesses remarkable clinical reliability given adequate testing compliance, as the results demonstrated reproducibility among the control participants as well as their patient counterparts. In addition, we propose that this handheld system has promising potential in testing patients where the clinical ERG cannot be easily implemented such as in resource-limited centers that do not have established visual electrophysiology units.

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Compliance with ethical standards

Conflict of interest The author declares that they have no conflict of interest.

Statement of human rights All research procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Statement on the welfare of animals This article does not contain any studies with animals.

Informed consent Informed consent was obtained from all participants included in the study.

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