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# Rod a-wave analysis using high intensity flashes adds information on rod system function in 25% of clinical ERG recordings

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ABSTRACT

*Purpose:* To investigate whether rod a-wave analysis using high intensity flashes adds information above that obtained with standard ERG.
*Methods:* A total of 2396 eyes were recorded. Patient age was 2.4 months–84.6 years.
*Results:* A-wave analysis of high intensity flashes provided additional information on rod system function in 25% of eyes recorded, most importantly in subjects with midretinal disease and artificially reduced rod responses. High intensity flashes also provided measurable responses for longitudinal monitoring in rod dystrophies with non-recordable rod ERGs.
*Conclusions:* Clinical ERG testing would benefit greatly from adding high intensity flashes to its standard testing conditions.



## 1. Introduction

Assessing rod photoreceptor function is one of the main objectives in visual electrophysiological testing of the retina. Current ISCEV (International Society for Clinical Electrophysiology of Vision) recommendations for minimal standard clinical ERG (electroretinogram) includes one step with low intensity flashes under scotopic conditions, giving a b-wave response called the rod ERG (Marmor, Holder, Seeliger, & Yamamoto, 2004). For investigators wishing to explore this response in more detail, b-waves obtained to a series of flash intensities can be described by the Naka–Rushton equation (Fulton & Rushton, 1978; Arden, Carter, Hogg, et al., 1983), in which $V_{max}$ represents the maximum b-wave amplitude obtained before cone intrusion appears, and $K$ (or $\log K$) represents the light intensity required to produce 50% of $V_{max}$.

There are several reasons why the scotopic b-wave response may not be the optimum measure of rod activity. (1) Even though the rod ERG is a rod photoreceptor driven response, the b-wave itself is generated from the midretina, representing activity from mainly bipolar cells (Green & Kapousta-Bruneau, 1999). In disorders affecting midretinal function, the scotopic b-wave may consequently be abnormal, or even non-recordable, although the rod photoreceptors might have normal function. (2) In some cases, scotopic b-wave responses can be very difficult to record reliably. Such cases include patients having difficulty complying with ERG testing, e.g. young children or patients with considerable blinking. Variable Bell's phenomenon may artificially reduce retinal illuminance in patients with ERG recordings performed under sedation or general anaesthesia, which can also be the case for patients with cataracts, giving artificially decreased rod ERG responses. (3) The rod ERG has a very wide normal distribution and separating normal results from abnormal may be difficult (Birch & Anderson, 1992). (4) In infants, the lower retinal sensitivity makes recording of rod ERG difficult or impossible (Fulton, Hansen, & Westall, 2003; Fulton et al., 2006).

Hood and Birch have suggested an alternative method to assess rod function with high intensity flashes (Hood & Birch, 1996). The leading edge of the scotopic a-wave has been shown to represent rod photoreceptors (Jamison, Bush, Lei, & Sieving, 2001), and by subtracting photopic high intensity flash responses from scotopic high intensity flash responses, rod photoreceptor function can be isolated. A-Wave analysis of high intensity flashes has been referred to as the bright flash ERG, although there is currently some confusion with regards to this terminology since the word bright is non-quantitative, and depending on the context, used to describe a wide range of flash intensities. Hood and Birch (1996, 2006) have demonstrated that the flashes used for a-wave analysis of rod photoreceptor function needs to be sufficiently intense to elicit (a close to) maximal receptor response. The medium-intense flash of approximately 10 cd s/m$^2$ suggested in the current ISCEV ERG standard (Marmor et al., 2004) may thus not be an adequate measure of

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rod photoreceptor function. Two recent publications from Marmor, Serrato, and Tzekov (2003) and Marcus, Cabael, and Marmor (2006) have argued that in a routine clinical setting, high intensity a-wave analysis of photoreceptor function does not add essential information above that obtained with standard ERG. However, other authors have shown maximum rod photoreceptor response derived from high intensity flashes to be more reliable and clinically sensitive than the rod ERG (Vaegan & Narfstrom, 2008; Vaegan & Narfstrom, 2005).

Rod a-wave analysis of high intensity flashes has been a part of our routine clinical ERG protocol for more than 3.5 years. The aim of this paper is to compare information obtained with standard rod ERG to that obtained by high intensity flashes. We moreover report on groups of retinal disorders, as well as testing situations, in which rod a-wave analysis of high intensity flashes provided valuable information or helped with diagnosis, which may not have been achieved using only current standard ERG steps. We also report on rod photoreceptor function from infancy to old age in patients with normal standard ERG results.

## 2. Methods

### 2.1. Methods

High intensity flashes of 241 photopic cd s/m$^2$, given in both scotopic and photopic conditions, were added to our clinical protocols in March 2004. ERGs recorded from March 2004 to October 2006 were obtained using the UTAS 3000 system (LKC Technologies, Gaithersburg, MD). ERGs recorded from November 2006 to October 2007 were obtained using the Espion ColorDome system (Diagnosys LLC, Lowell, MA). Prior to testing pupils were fully dilated using 1% tropicamide and 2.5% phenylephrine, and bipolar Burian–Allen contact lenses were placed in one or both eyes after administration of 0.5% proparacaine drops. A ground electrode was attached to the forehead. Scotopic steps were recorded after a minimum of 30 min dark adaptation. Photopic steps were recorded after 10 min of light adaptation on a white background of 29 photopic cd m$^{-2}$. The short protocol includes nine steps and the extended protocol 14–15 steps, both including the two high intensity steps. The extended protocol includes seven to eight scotopic steps with intensity lower than the standard flash for Naka–Rushton analysis. Both protocols include all conditions required for ISCEV standard ERG (Marmor et al., 2004). The extended protocol was always chosen if possible. The short protocol was used for recordings in non-sedated young children, patients with non-recordable or very attenuated scotopic b-waves, and patients having difficulties complying with ERG testing. The multiple responses to each condition were averaged after rejection of trials containing large artefacts. For high intensity a-wave analysis, three flashes were given with 30 s inter-trial delay in scotopic conditions, and eight flashes with 2 s inter-trial delay were given in photopic conditions.

Analysis of a-wave responses to high intensity flashes to obtain surrogate measures representing rod photoreceptor sensitivity ($S$) and maximum response ($R_{max}$) was performed using in-house produced software based on the simplified model according to Hood and Birch (1996, 2006). The software digitally subtracts the photopic response from the scotopic response. Rod photoreceptor maximal response ($A_{max}$) is obtained by fitting a horizontal line to the trough of the subtracted trace (amplitude measured from baseline at time 0). For rod photoreceptor sensitivity, the program identifies the steepest slope of the a-wave of the subtracted trace (within 30 ms after the flash), and $t_{Amax}$ is obtained by extrapolating a line fitted to this leading edge of the a-wave (Fig. 1). The a-wave fit was always examined by eye and could be modified in case of erroneous results of the fitting algorithm.

Naka–Rushton analysis was performed using LKC software.

### 2.2. Subjects

All patients referred for ERG testing between March 2004 and October 2007 were reviewed. In patients where both eyes where tested, the testing was done for both eyes at the same time in all but very few cases. If a patient had consecutive ERGs, each testing session was reviewed as a separate episode.

The study was approved by the Research Ethics Board at the Hospital for Sick Children and followed the tenets of the Declaration of Helsinki.

## 3. Results

A total of 1521 full-field ERG recordings were performed between March 2004 and October 2007, and high intensity flashes were included in 1337 of them. These 1337 recordings (2396 eyes) were performed in 1049 patients and patient age ranged from 2.4 months to 84.6 years (mean 19.3 years, standard deviation 20.5 years). Results from eyes with diverging results for rod ERG and high intensity rod a-wave analysis, as well as developmental results for a group of patients with normal standard ERG, are presented below. Results from eyes with converging results for both rod ERG and high intensity rod a-wave analysis (i.e. diminished–diminished) are not presented in detail below.

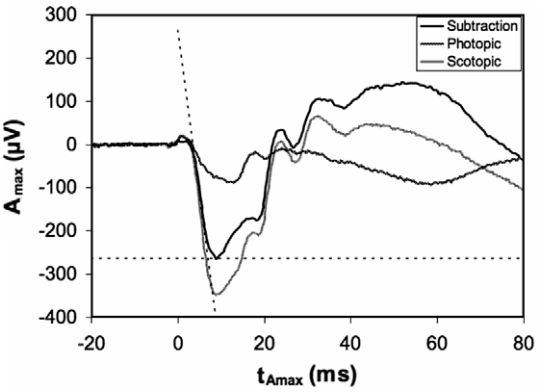


**Fig. 1.** The example shows the typical wave morphology of a normal response to high intensity flashes. The photopic response is subtracted from the scotopic response, and rod photoreceptor function is derived from the subtracted trace.

### 3.1. Rod photoreceptor function in patients with normal ERGs

All patients with normal ERG results recorded with the UTAS 3000 system were selected. Results were limited to only one of the ERG systems in order to minimize system dependent variation. Standard ISCEV ERG results within normal limits for age were found in 433 eyes of 269 patients, with an age range of 3.4 months to 82 years (mean 20.6 years, standard deviation 20.2 years). The results for rod photoreceptor maximal response ($A_{max}$) and sensitivity ($t_{Amax}$) in these patients are shown in Fig. 2a and b. For patients under 10 years of age the results are shown in more detail in Fig. 3a and b. For ages 5–82 years, results for both $A_{max}$ and $t_{Amax}$ were very stable and showed only minor decline with age. For amplitude, the best fit regression line gives a decrease of 0.0001 log $A_{max}$/year. For sensitivity, the best fit regression line gives a decrease in sensitivity of 0.0003 log $t_{Amax}$/year.

### 3.2. Non-recordable rod ERG and normal $A_{max}$

In 56 eyes of 40 recordings, the rod ERG was nonrecordable above noise while $A_{max}$ was within normal limits. In nine eyes (5 patients), rod photoreceptor sensitivity was diminished and sequential testing in 3/5 patients revealed a progressive rod-cone dystrophy. Diagnoses in the group with non-recordable rod ERG and normal rod photoreceptor response ($A_{max}$ and $t_{Amax}$ within normal limits) included CSNB, X-linked retinoschisis, early rod-cone dystrophy, ESCS (Enhanced S-cone Syndrome) and Birdshot retinochoroidopathy.

### 3.3. Diminished rod ERG and normal $A_{max}$

In 188 eyes of 171 recordings, the rod ERG amplitude was below normal limits for age, while $A_{max}$ was within normal limits. For 103 (55%) of these eyes, the patient was diagnosed with a midretinal disorder such as CSNB, Birdshot retinochoroidopathy, children with Infantile Spasms being monitored for

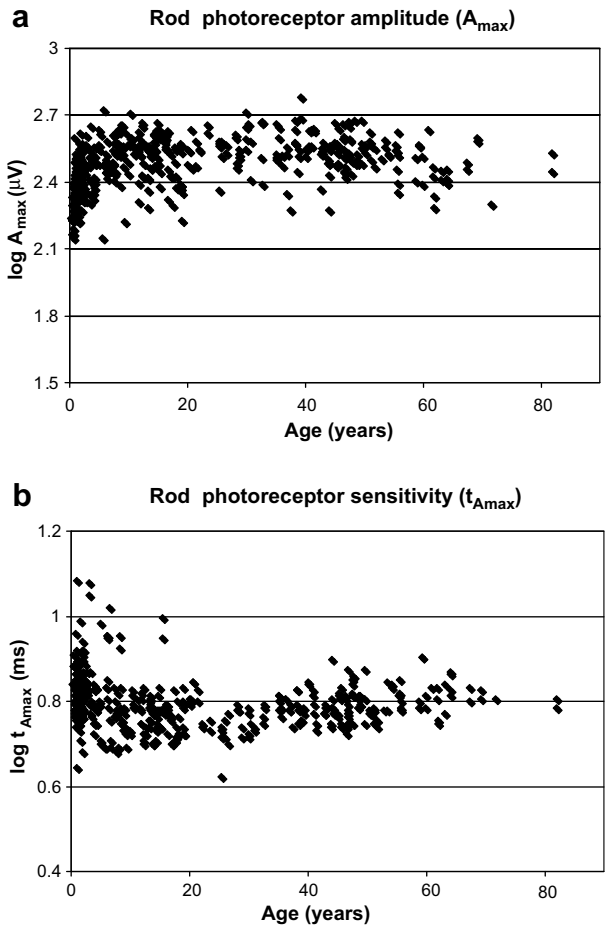


**Fig. 2.** Results for rod photoreceptor maximal amplitude, $A_{max}$, (a) and sensitivity, $t_{Amax}$, (b) for all patients with normal standard ERG results. Results for patients over 5 years of age: mean amplitude 340.7 μV (standard deviation 70.4 μV; 95% CI 199.9–481.5 μV); mean sensitivity 6.17 ms (standard deviation 0.88 ms; 95% CI 4.42–7.93 ms).

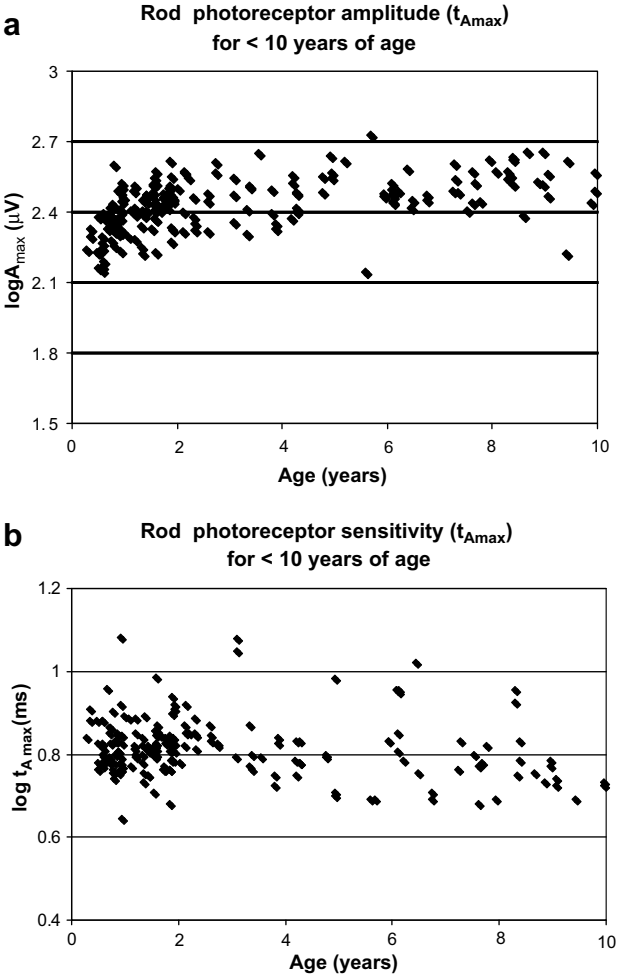


**Fig. 3.** Results for rod photoreceptor maximal amplitude, $A_{max}$, (a) and sensitivity, $t_{Amax}$, (b) for patients <10 years of age with normal standard ERG results.

vigabatrin toxicity and patients with XL retinoschisis. For the remaining eyes, patient diagnoses included cone-rod dystrophies, (early) rod-cone dystrophies, AZOOR (Acute zonal occult outer retinopathy), mitochondrial disorders and undiagnosed retinal dysfunction.

### 3.4. Non-recordable rod ERG and diminished $A_{max}$

In 282 eyes of 170 recordings, the rod ERG was non-recordable above noise, while a diminished rod photoreceptor response could be recorded using rod a-wave analysis of high intensity flashes. This group mainly consisted of rod-cone and cone-rod dystrophies.

### 3.5. Unreliable rod ERG and reliable response to high intensity flashes

In 86 eyes of 64 recordings the rod ERG results were unreliable, most of these patients had notes of excessive blinking or Bell's phenomenon in the report. In 82 of these eyes, reliable responses to high intensity flashes could be recorded, 76 of which were within normal limits.

### 3.6. Recordable rod ERG and unreliable response to high intensity flashes

In 21 eyes of 15 recordings with rod ERG amplitude of at least 15 μV (five eyes in three subjects, with rod ERG amplitude marked at <15 μV, were considered non-recordable above noise, and were excluded from this group), the rod photoreceptor response could not be calculated due to unreliable scotopic and/or photopic high intensity recording. The failed high intensity flash responses were mostly due to poor patient compliance, but in two patients due to technical problems (massive 60 Hz intrusion).

In total, rod a-wave analysis using high intensity flashes gave additional information regarding rod system function, above that obtained by only rod ERG, in 608 (25%) of the 2396 eyes recorded (Table 1).

### 3.7. Naka–Rushton analysis of scotopic b-wave response

It was possible to fit the Naka–Rushton equation in 63 out of the 264 eyes with normal $A_{max}$, and either unreliable or diminished rod ERG. Results for Naka–Rushton analysis were normal in nine eyes, eight of these had unreliable rod ERG results with notes of blinking or Bell's phenomenon in the report, and one eye had a cataract of unknown density.

### 3.8. Detailed analysis of a midretinal disorder: CSNB as an example

Standard ISCEV ERG results with typical electrophysiological features of either complete CSNB or incomplete CSNB, and normal rod photoreceptor function ($A_{max}$ and $t_{Amax}$), were found in 29 patients. All but 6 patients had at least two ERGs performed to

**Table 1**
Eyes with diverging results for rod ERG and rod a-wave analysis using high intensity flashes

| Rod ERG response (0.0063 cd s/m$^2$) | Rod photoreceptor response (241 cd s/m$^2$) | Number of eyes | Rod a-wave analysis using high intensity flashes gave additional info on rod system function | Examples of conditions |
|---|---|---|---|---|
| Nonrecordable | Within normal limits | 56 | * | CSNB, Birdshot retinochoroidopathy |
| Nonrecordable | Diminished | 282 | * | Rod-cone dystrophies |
| Nonrecordable | Nonrecordable | 281 | | |
| Diminished | Within normal limits | 188 | * | Midretinal disorders and artificially reduced rod ERG responses |
| Diminished | Nonrecordable | 21 | | |
| Unreliable | Within normal limits | 76 | * | Bell's phenomenon, blinking |
| Unreliable | Diminished | 6 | * | Rod-cone dystrophies |
| Unreliable | Unreliable | 4 | | |

Total number of eyes recorded: 2396 (all eyes are not represented below).

explore the possibility of a progressive condition (Table 2). In two patients with standard ERG results consistent with complete CSNB, rod a-wave analysis using high intensity flashes showed slightly diminished $A_{max}$ and normal rod photoreceptor sensitivity, sequential testing confirmed a stationary condition most consistent with complete CSNB (Row 3, Table 2).

In two patients referred for ERG with clinically suspected CSNB, results from the first standard ERG were consistent with an early rod dysfunction and rod a-wave analysis using high intensity flashes showed slightly diminished $A_{max}$ and normal rod photoreceptor sensitivity (Row 4, Table 2). In both these patients, sequential ERG testing was within normal limits.

In five patients referred for ERG with clinically suspected CSNB, results from the first ERG were consistent with a rod-cone dysfunction. All had clearly diminished rod photoreceptor $A_{max}$ and 2/5 had decreased rod photoreceptor sensitivity. Sequential testing confirmed a progressive rod-cone dystrophy in two patients, two patients had a family history of rod-cone disease and one patient had a very attenuated ERG.

There were no patients with a standard ERG suggestive of CSNB with a non-recordable response to high intensity flashes.

## 4. Discussion

In clinical ERG recordings, information on rod function is usually derived from the scotopic b-wave response, commonly called the rod ERG (Marmor et al., 2004). This study shows that in certain diseases, such as midretinal disorders, as well as in artificially reduced scotopic b-wave responses, a-wave analysis of high intensity flashes is a superior method for obtaining results for rod photoreceptor function.

Previous reports by Marmor et al. (2003) and Marcus et al. (2006) have concluded that high intensity flashes add little diagnostic information above that obtained with standard ERG steps. Both reports, however, used much smaller sample sizes than the current study, neither study had any group with midretinal disorders and none of their patients with rod-cone disease had a nonrecordable rod ERG. Moreover, the paper by Marcus et al. (2006) compares high intensity flash a-wave results with ISCEV standard flash a-wave results. The a-wave of the ERG represents photoreceptor function, and it could be argued that the scotopic standard flash a-wave could be used as a marker of rod photoreceptor function. At light intensity levels where a-waves appear in scotopic conditions there is, however, substantial cone contribution, and the a-wave elicited by the scotopic standard flash is a mixed rod-cone response. Moreover, the a-wave amplitude of a moderate intensity flash does not represent the peak photoreceptor response since the development of the b-wave (the second order neurons response) interrupts the a-wave (Hood & Birch, 1996).

Unreliable scotopic b-waves (rod ERG) caused by blinking is a real problem in a substantial number of patients in any clinical ERG lab. In subjects with excessive blinking, scotopic b-waves may be very difficult to record reliably since the timing of the myoclonic reflex (blink reflex) coincides with the b-wave response (Nilsson et al., Submitted for publication). High intensity flash a-waves have an implicit time of 5–10 ms, making this response less prone to blink intrusion. In our study a reliable high intensity flash response could be recorded in 82 out of 86 (95%) eyes with unreliable rod ERG results. Even when reliable rod ERG responses can be obtained, separating normal from abnormal results may be difficult. Rod ERG responses have a very wide intersubject variability (Birch & Anderson, 1992), with a coefficient of variation (one standard deviation divided by the mean) at 43% for adult control subjects (Westall lab 2008). The range of normal results is consequently very wide and there is likely significant overlapping between normal and abnormal results. For rod photoreceptor $A_{max}$, the coefficient of variation in this study was 20% for adult control subjects.

Previous work has shown rod photoreceptor $A_{max}$ to be virtually stable across 5–75 years of age (Birch, Hood, Locke, Hoffman, & Tzekov, 2002), making it a good marker for longitudinal studies of rod photoreceptor function. Our results from patients age 3.4 months to 82 years with normal standard ERG results show a maturation effect up to age 3–4 years (which is in concordance with Fulton et al. (2003)), where after $A_{max}$ remains stable up to 82

**Table 2**
Results for patients with standard ERG results consistent with CSNB, or with clinically suspected CSNB

| Number of patients | First standard ERG most consistent with | Rod photo-receptor $A_{max}$ | Rod photo-receptor $t_{Amax}$ | Final electrophysiological phenotype (number of patients with at least 2 ERGs in parenthesis) |
|---|---|---|---|---|
| 19 | Incomplete CSNB | WNL | WNL | Incomplete CSNB (16/19) |
| 10 | Complete CSNB | WNL | WNL | Complete CSNB (7/10) |
| 2 | Complete CSNB | Slightly below normal limits | WNL | Complete CSNB (2/2) |
| 2 | Rod dysfunction | Slightly below normal limits | WNL | Sequential ERG testing WNL (2/2) |
| 3 | Rod-cone dysfunction | Diminished | WNL | Progressive rod-cone dystrophy (1/3) |
| 2 | Rod-cone dysfunction | Diminished | Decreased | Progressive rod-cone dystrophy (1/2) |

WNL, Within normal limits for age.

**Table 3**
Right eye results over five consecutive ERGs for a patient with Retinitis Pigmentosa

| Date of ERG | Rod ERG amplitude (67–244 μV) | Rod b-wave implicit time (105–133 ms) | Standard flash (sc) a-wave amp (143–373 μV) | Standard flash (sc) a-wave it (18–24 ms) | Standard flash (sc) b-wave amp (370–739 μV) | Standard flash (sc) b-wave it (40–54 ms) | Rod photo-receptor $A_{max}$ (200–482 μV) | Rod photo-receptor $t_{Amax}$ (4.4–7.9 ms) |
|---|---|---|---|---|---|---|---|---|
| May 2002 | 41* | 132 | 178 | 26* | 271* | 55* | N/A | N/A |
| July 2003 | NR | NR | 104* | 27* | 99* | 55* | N/A | N/A |
| Aug 2004 | NR | NR | 100* | 27* | 102* | 53 | 187* | 7.2 |
| Aug 2005 | NR | NR | 61* | 29* | 28* | 48 | 122* | 8.6* |
| June 2006 | NR | NR | 60* | 29* | 28* | 47 | 76* | 7.7 |

| Date of ERG | Naka–Rushton *V*max (372–646) | Naka–Rushton logK (−2.91–−2.11) | Naka–Rushton *n* (0.7–1.4) |
|---|---|---|---|
| May 2002 | 265* | −1.4* | 0.6* |
| July 2003 | Not possible | Not possible | Not possible |
| Aug 2004 | Not possible | Not possible | Not possible |
| Aug 2005 | Not possible | Not possible | Not possible |
| June 2006 | Not possible | Not possible | Not possible |

Lab normal ranges for responses within parenthesis, results marked with * are considered abnormal responses.
Note the results for rod a-wave analysis using high intensity flashes giving longitudinal information on the progressive rod photoreceptor dysfunction even though rod ERG (scotopic b-wave) is non-recordable, Naka–Rushton analysis not possible and standard flash response variable. Rod ERG = 0.0063 cd s/m$^2$, standard flash = 2.3 cd s/m$^2$, rod photoreceptor = 241 cd s/m$^2$.
NR, non-recordable above noise; N/A, not available.

years-of-age (Fig. 2). Even though neither Birch et al. (2002) nor the current study included any information on cataract in the older population, results showing stable $A_{max}$ across older ages suggest that rod photoreceptor may be less sensitive to low-medium grades of cataract than scotopic b-wave responses (Birch & Anderson, 1992). This finding may, however, in part be due to the use of white flashes and computer-subtraction of cone components rather than the use of short-wavelength flashes (which are more influenced by cataract) in the older literature.

A-wave analysis of high intensity flashes has, moreover, been shown to have low test–retest variability when compared with rod ERG response. For rod ERG responses, up to 46% decrease in amplitude could be contributed to intervisit variability, while for rod photoreceptor maximal amplitude a 23% decrease has been reported being a statistically significant change (Birch et al., 2002). In our study, 258 eyes with non-recordable rod ERG (11% of the total number), had a recordable response to high intensity flashes. In this group, mainly consisting of rod-cone and cone-rod dystrophies, a-wave analysis of high intensity flashes does not add any critical diagnostic information, but a measurable rod photoreceptor response may be very valuable for longitudinal monitoring of disease progression (Table 3). In 6 eyes the rod ERG was unreliable, but a diminished $A_{max}$ could be recorded with high intensity flashes.

Naka–Rushton analysis may add information on rod function in a number of conditions; then again, similar to rod ERG, the information is based on the activity of second order neurons. Similarly again, in disorders where scotopic b-waves are reduced due to midretinal disorders or unreliable due to low compliance, Naka–Rushton may be impossible to calculate, or may give subnormal results even though the rod photoreceptors may have normal function. Out of the 264 eyes in our sample with subnormal or unreliable rod ERG and normal rod photoreceptor $A_{max}$ results, adding the Naka–Rushton protocol (10 minutes testing) to the standard ISCEV ERG would only have indicated normal rod function in 9 eyes, 8 of which had unreliable rod ERG due to blinking or Bell's phenomenon and one patient with a cataract.

An interesting group in this study, in which high intensity flashes seem to be of particular importance, were subjects with midretinal disorders. Our results show that the rod ERG may be diminished or even non-recordable, even though a-wave analysis of high intensity flashes indicate normal rod photoreceptor function. In our study, all subjects with CSNB (incomplete as well as complete) had normal rod photoreceptor function as measured by a-wave analysis of high intensity flashes (two subjects had $A_{max}$ results just below lower limits of normal). Subjects with clearly subnormal $A_{max}$ and/or decreased sensitivity at rod a-wave analysis of high intensity flashes were all found to have progressive retinal disorders (Table 2).

Marmor et al. (2003) and Marcus et al. (2006) argue that the a-wave analysis of high intensity flashes cannot be generally recommended to the visual electrophysiology community, since it requires complicated computing and signal analysis (subtraction of two responses and fitting the leading edge of the a-wave of the obtained trace). This may be true for labs with very limited technical support, however, most ERG systems allow export of data to Excel where the subtraction can be performed in ASCI file format and the measurement performed with pen and ruler (Hood & Birch, 2006). Moreover, if a demand should arise, ERG manufacturers could easily incorporate a sequence that automatically calculates rod photoreceptor $A_{max}$ and $t_{Amax}$ into the software. For clinical purposes, a protocol with a single high intensity flash has been shown sufficient and adds less than two minutes testing to the current ISCEV standard ERG recommendations (Hood & Birch, 1996; Nilsson et al., Submitted). We have in another study demonstrated that 241 photopic cd s/m$^2$ is sufficiently bright to give a saturating rod photoreceptor response (Johnson & Massof, 1982).

In conclusion, rod a-wave analysis of high intensity flashes added information on rod system function above that which was provided by the rod ERG in 25% of recorded eyes (Table 1). High intensity flashes were especially valuable in suspected midretinal disorders and patients with artificially reduced rod-isolated responses, and can facilitate longitudinal monitoring of rod function in rod dystrophies. In our experience, rod a-wave analysis of high intensity flashes is a valuable tool for assessing rod system function in a wide set of retinal diseases and testing situations. Standard clinical ERG would benefit greatly from adding rod a-wave analysis of high intensity flashes to its testing conditions.

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