Automated Light- and Dark-Adapted Perimetry for Evaluating Retinitis Pigmentosa: Filling a Need to Accommodate Multicenter Clinical Trials

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Purposes. The purpose of this study was to develop a convenient means to measure rod (and cone) function by automated perimetry in patients with inherited retinal degenerations (IRDs).

Methods. A currently available automated perimeter was used to determine sensitivity (in decibels) to a blue target in the dark-adapted (DA) state and a white target in the light-adapted (LA) state. Normal subjects and IRD patients were evaluated with a full-threshold 71-locus strategy (the retinitis pigmentosa [RP] test) and a size III target. Comparisons were made with results from the more commonly used methods of two-color DA perimeter and middle/long-wavelength LA perimeter in the same patients.

Results. Rod function using the blue target and the RP test was determined for normal subjects by measuring DA sensitivities. If patients detected the blue stimulus in the DA state, it was determined whether the value was rod mediated by using normal data acquired during the cone plateau phase of dark adaptation. If rod mediated, rod sensitivity loss (RSL) was calculated and mapped across the visual field. Light-adapted sensitivities in normal subjects were also measured, permitting cone sensitivity losses (CSL) to be calculated for the patients. Multiple methods were used to compare RSL and CSL results with those from two-color DA perimeter and chromatic LA perimeter, and there was close correspondence between the methods.

Conclusions. The unmodified automated static perimeter used in the DA and LA states presents a practical approach to accomplish current goals of treatment trials in IRDs. This proof-of-principle study is an initial step toward establishing a clinical method to gather reproducible data on photoreceptor-mediated sensitivity.

Keywords: cone, rod, retinal degeneration
reassessed the feasibility of DA automated perimetry with a commercially available instrument, designed protocols that could be used in most ophthalmology clinics, established normal limits for the protocols, examined a series of patients with IRDs for feasibility of technique, and compared the results with those from a published method.15

**METHODS**

**Human Subjects**

Eighteen IRD patients (age, 24–66 years) were included (Table). Patients had a complete eye examination. A group of normal subjects (n = 11; age, 22–57 years) were also studied. Informed consent was obtained; procedures followed the Declaration of Helsinki, and there was institutional review board approval.

**Localised Visual Sensitivities**

Protocols determined rod- and cone-mediated visual sensitivities at 71 loci on a 12° grid across the visual field: extending from 72° temporally, 48° nasally, 36° superiorly, and 48° inferiorly. Foveal sensitivity was determined using a diamond-shaped array of light emitting diodes as the fixation target. This series of 71 loci will be referred to as the retinitis pigmentosa (RP) test. Two strategies were used, both with full-threshold testing: first, our previously published method,15,21 and second, the new method that is the topic of the current work.

**RESULTS**

**Determining Regional Rod- and Cone-Mediated Function With a Commercially Available Automated Perimeter**

To assay the regional variation of rod function across the visual field with a method that could have widespread clinical use,
we altered our previous strategy of two-color DA static perimetry. Our original method required modifications of the HFA that involved optical and mechanical engineering so that short- and long-wavelength interference filters and neutral density filters could be inserted into the optical pathway of the light stimulus. This has been both feasible and useful for characterizing the regional variation of rod- and cone-mediated function in IRD patients. As the HFA models advanced technologically, we revised our modifications to accommodate each new generation of instruments.

The new method to assay rod function, however, uses an unmodified HFA, permitting DA perimetry to be performed on IRD patients in a clinic that would otherwise be using LA perimetry for patients with other eye diseases that require automated perimetry, such as glaucoma. Numerical DA blue sensitivity results for the RP test strategy in normal subjects (displayed as right eye data) are shown (Fig. 1A). Locus-by-locus mean results (Fig. 1A, left) and lower limits of normal (Fig. 1A, right) are given. In general, mean sensitivity to this blue target is relatively flat (~45–55 dB) across the field sampled; sensitivity at fixation is lower. The results compare favorably with DA sensitivities to the short-wavelength target in normal subjects using the modified HFA and the larger target. To define for each locus the lowest sensitivity level that can be ascribed to rod-mediated function, in normal subjects, we determined DA blue sensitivity at cone plateau, that is, after a period of light adaptation known to desensitize rods and before rods recover (lower inset to the right of Fig. 1B). The mean sensitivity results for the RP test (blue stimulus, size III) at cone plateau in a subset of normal subjects are shown (Fig. 1B). The DA sensitivity of a patient is considered to be detected by rods if it falls above the lower limit of normal DA blue at cone plateau.

For LA perimetry (10 cd/m² white background light), the achromatic (white) stimulus is used with the size III target. This is a common strategy in glaucoma monitoring, and such increment thresholds with automated perimetry have also been used in clinical trials of IRDs. Once the sensitivities to the blue stimulus are recorded in the DA state in an IRD patient, questions about the results arise: Are there any rod-mediated loci? If so, is rod function normal or abnormally reduced? If reduced, by how much? The LA sensitivities to a white stimulus need less interpretation and sensitivity loss (CSL), the difference between normal mean and patient results at each locus, ranged from no loss to 19 dB (average, 5.9 dB) for the 30 loci with detectable LA function. In summary, the technique in this patient shows detectable but mostly abnormal rod function in the inferior field and less affected cone function in this same region; there is a gradient of rod and cone function from more severe near the scotoma to less severe with eccentricity into the inferior field.

Patient 1 is a 24-year-old man with X-linked RP (RPGR mutation). With DA perimetry and the blue stimulus, there were only eight extrafoveal loci detected, and these were in the nasal and temporal periphery (Fig. 3A). All of these peripheral loci were rod mediated (Fig. 3B); RSL ranged from 8 to 24 dB (average, 14.9 dB). With LA perimetry, there were 19 detectable extrafoveal loci, mainly in the nasal and temporal periphery. Kinetic perimetry with the III-ie target showed a central island and patches of nasal and temporal periphery with a complete annular midperipheral scotoma. Cone sensitivity loss ranged from 4 to 27 dB (average, 17.2 dB). Sensitivity at the foveal locus was also reduced. In summary, DA perimetry in this patient shows detectable but abnormal rod function only present in the peripheral field; cone function, also abnormal, is present in these peripheral regions as well as centrally.

Comparing Results of the New Methodology With Those of Our Standard DA and LA Chromatic Perimetry in the Same IRD Patients

Of the 18 IRD patients examined with both DA perimetry techniques, a group of seven patients had >25 rod-mediated loci (average, 55 loci) as determined by the standard two-color method at the 70 extrafoveal loci of the RP test (Fig. 4A). The remaining 11 patients had ≤11 rod-mediated loci (average, 3.7 loci). The first group provided the opportunity to ask whether the rod-mediated loci with the standard method were all detected by the new method of DA perimetry. The answer was that most were, but some were not (Table). For example, the new DA method detected as rod mediated >95% of loci detected by the standard method in five patients (P18, P16, P15, P11, and P3). Two patients, P13 and P17, had detection of 79%. We tested the hypothesis that detected and undetected rod-mediated loci differed in degree of RSL (Fig. 4A). Mean RSL was different for detected and undetected loci in all patients (P < 0.05, t-test), with undetected loci having greater RSL. Mean differences between subsets ranged from 8.2 to 35 dB (average, 24 dB).

We next analyzed the relationship between the results of the two methods (DA-500 nm versus DA-blue; LA-600 nm versus LA-white). Included in the analysis were 18 patients. All subjects were tested by both methods on the same day. For DA data, we only included locations with rod mediation (as determined by our standard method). Linear relationships between methods were evident for both DA and LA datasets (R² = 0.90 and 0.87, respectively; P < 0.01, F-test; Fig. 4B). There were shifts of scale of ~5.2 dB for DA and ~4.8 dB for LA (mean difference, DA-500 nm minus DA-blue and LA-600 nm minus LA-white, respectively); this is not shown in the figure: 95% limits of agreement between methods were ±9.9 and ±6.8 dB for DA and LA data (Fig. 4C), respectively. As a reference, our previously reported intravisit variability value for DA static perimetry would correspond to limits of agreement for a difference of measurements of ±7.5 dB (2
**FIGURE 1.** Normal data for DA and LA RP tests using the unmodified HFA. (A) Dark-adapted blue normal results. *(Left)* Mean normal. *(Right)* Lower limits of normal (mean – 2 SD). *(B)* Dark-adapted blue at cone plateau. *(Left)* Mean normal. *(Right)* Dark adaptation functions indicating with brackets the concept of final DA thresholds *(upper)* and at cone plateau *(lower).* *(C)* Light-adapted white normal results. *(Left)* Mean normal. *(Right)* Lower limits of normal (mean – 2 SD).
**FIGURE 2.** Patient data using DA and LA RP tests. Results are from the right eye of a patient with autosomal dominant RP caused by a *rhodopsin* mutation (P15). (A) Dark-adapted blue results. (B) Loci determined to be rod mediated, R. (C) Rod sensitivity loss at the identified rod-mediated loci. (D) Rod sensitivity loss map; grayscale shown below and to the right. (E) Light-adapted white results. (F) Kinetic visual field with III-4c target. (G) Cone sensitivity loss at the loci with detectable LA function. (H) Cone sensitivity loss map; grayscale shown below and to the right. N, nasal; T, temporal; I, inferior; S, superior visual field. x, physiologic blind spot locus. F, foveal-fixation locus.
FIGURE 3. Patient data using DA and LA RP tests. Results are from the right eye of a patient with X-linked RP caused by an RPGR mutation (P1). (A) Dark-adapted blue results. (B) Loci determined to be rod mediated, R. (C) Rod sensitivity loss at the identified rod-mediated loci. (D) Rod sensitivity loss map; grayscale shown to the right. (E) Light-adapted white results. (F) Kinetic visual field with III-4e target. (G) Cone sensitivity loss at the loci with detectable LA function. (H) Cone sensitivity loss map; grayscale shown to the right. N, nasal; T, temporal; I, inferior; S, superior visual field. x, physiologic blind spot locus. F, foveal-fixation locus.
**FIGURE 4.** Comparison of methodologies. (A) Boxplots of DA data from 7 patients, each with at least 25 loci determined to be rod mediated by standard two-color perimetry. The questions asked are how many of these loci are detected as rod mediated by the new DA method, and, when there are undetected loci, whether there is any difference in RSL in detected (white boxplots) versus undetected (gray boxplots) ones. Numbers within the graphic below boxplots are the detected versus undetected loci counts; the sum of the two numbers represents the total number of rod-mediated loci detected by the standard two-color perimetry. Band inside the box is the median; bottom and top of box are the first and third quartiles. Ends of whiskers represent the 10th and 90th percentiles. (B) Relationship of results of the two DA and the two LA methods. (C) Limits of agreement (95%) between the two DA methods and between the two LA methods. Mean differences of $-3.2$ dB for DA-500 nm minus DA-blue and $-4.8$ dB for LA-600 nm minus LA-white originating from differences of the definition of maximum (0 dB) stimulus have been removed from the plots shown in B and C.
SD variability limits, ± 5.3 dB for a single measurement, DA-white, size V33.

From DA and LA Perimetry Data Collection to Data Processing: A Plan

The many conventionally used tests in automated perimetry (e.g., for glaucoma) have instrument-based analyses. For the DA (and LA) testing we devised for IRD patients, the unmodified HFA is used solely to collect the data. The HFA 750i can be configured to export data from a test immediately after completion (Save and Transmit option) or at any time afterwards using the Transfer option within the File menu. In models having USB functionality, the exported files are written on a USB stick; for older models, they can be transferred using an RS232C to USB serial adapter. Either the memory stick or the serial adapter can be connected to a USB port in a standard PC (Windows, Mac, or Linux) or a mobile device (Android, IOS). Once in the laptop or mobile device, a downloadable program or app would read the files for rod and cone function analysis. In future multicenter clinical trials, the files can optionally be sent over the internet to a remote site for analysis or storage. In that case, the files would be coded for anonymization, digitally signed for integrity, and encrypted for privacy prior to data transfer (Fig. 5A).

Rod and cone visual function analysis is shown as a flow diagram (Fig. 5B) and can run locally on a PC or mobile device or remotely using a web-accessible application. Data as received from the perimeter are parsed, and the following algorithm is applied for each location. First, a single sensitivity estimate is obtained for the location (by averaging, in the case of locations with multiple samples); second, sensitivity losses are obtained by subtracting the subject’s sensitivity from corresponding mean normal sensitivity. For RSL, the measured sensitivity would first be determined to be from the rod system (far right branch of the diagram; Fig. 5B). This is necessary because DA perception at threshold may be mediated by cones in some cases. This assessment involves comparing the DA sensitivity against location-specific data for DA cone sensitivity (our DA results with the blue target at cone plateau). If the subject’s DA sensitivity is higher than this value, perception at this location is assumed to be mediated by rods, and RSL is calculated by subtraction from mean DA normal sensitivity (for normal subjects, DA perception is mediated by rods through-out the retina). If the comparison fails, dark-adapted rod mediation cannot be ascertained, and no RSL value is calculated. A data visualization step summarizes the results (as exemplified in Figs. 2 and 3) including rod and cone sensitivities, sensitivity losses, and mediation. Multiple visit data can also be longitudinally compared with historical records separately for rods and cones.

In summary, Figure 5 tries to reinform the readers that the commercially available automated perimeter, designed for glaucoma testing mainly, does not have analyses in the menu to cope with rod function sensitivity losses and does not fill the needs of the clinician-investigators who are seeking to understand the vision of IRD patients. Therefore, the specific data for IRD patients must be collected by the automated perimeter and then transferred to an external computer (e.g., PC). The latter device would have a suite of programs that can perform the step-by-step analysis shown in Figure 5B. Such programs would perform the needed analyses for many future purposes; not only determine rod and cone sensitivities across the visual field but allow visualization of the display as grayscale maps and answer key questions about intervisit variability and interocular differences and compare serial data of patients involved in either natural history studies or clinical trials of treatment.

DISCUSSION

Rod function is able to be measured with many methods (Supplementary Table S1). The method used in the clinic should fit the reason for making the measurement. The full-field electroretinogram (ERG) is a traditional diagnostic test that sums rod function across the entire retina. When the full-field ERG was used in clinical trials of RP patients at many different disease stages, the difficulties of obtaining recordable rod signals forced use of mainly cone ERGs as outcomes.A need to develop special techniques for recording small cone signals also occurred. There have been attempts to use focal stimuli to elicit rod ERGs.6 Less commonly used assays with full-field stimulation are pupillometry, visual evoked cortical potentials (VECPs), and the full-field sensitivity test (FST). Visual evoked cortical potentials would be expected to elicit responses mainly from the central retina, considering the representation at the cortex. The FST, a psychophysical method, was designed specifically to detect the most sensitive retinal region (independent of location) and mainly in eyes with fixation abnormalities.

It has long been recognized that there is value in understanding regional variation of rod (and cone) function in retinal degenerations, but the current era of therapy has further emphasized the importance. There are therapies targeted to specific retinal regions and plans of treatment specifically for rod photoreceptors (e.g., Refs. 44 and 45). Not knowing the degree of rod functional impairment before introducing therapy and where in the retina this rod function is located (in diseases well known to have different patterns of visual loss) precludes understanding whether a patient is a candidate for enrollment in specific clinical trials and, if a candidate, whether the therapeutic outcome is achieved.

Traditionally, psychophysical measures of regional rod sensitivity have used relatively large targets such as with the Goldmann-Weekers adaptometer. Automated static perimetry, which is used routinely in most clinics as a method to detect regional variation in visual function, is most commonly performed in the LA state. The concept of DA static perimetry using chromatic stimuli to discriminate rod from cone function became a means to study and categorize RP and has remained a specialty test in a small number of clinics (e.g., Refs. 2, 12–16, and 47). Whether manual or automated perimeters are used, the goal for DA testing has been the same: to determine sensitivity levels of rod-mediated function at many loci in the visual field. What are the current alternatives to a computerized projection perimeter modified to deliver chromatic stimuli? There is an LED-based automated perimeter for dark-adapted two-color static perimetry (Medmont DAC) (Cidecian AV, et al. IOVS 2016;57:ARVO E-Abstract 131), and it has the advantage of loci that cover most of the visual field. Disadvantages are current availability and also the issue of not being able to perform LA perimetry with the same instrument; the large number of loci in the instrument outweigh any disadvantage of being less flexible than a projection perimeter with custom testing options.

Whereas most automated perimeters have algorithms to determine stability of fixation (e.g., blindspot monitoring) and also permit viewing of the patient’s eye as a means to monitor fixation and cooperation, a method that allows performing perimetry while viewing the fundus is the ultimate way to localize the stimulus to the desired position on the retina. Fundus perimetry, as first named, has a decades-long history of being used in the DA state for measuring rod function in forms of retinal degeneration. Newer generations of fundus perimeters, some named microperimeters, have been devised and used mainly for measuring LA function in maculopathies (e.g., Ref. 50). More recently, advances have occurred such that
FIGURE 5. A plan for data processing for the DA and LA static perimetry results. (A) The HFA automated perimeter produces an XML-formatted file per test containing sensitivity values: one for DA and another for LA conditions. These files are transferred to a PC or mobile device for local analysis. The analysis programs would produce a single-page report for visualization of RSL and CSL, with contents similar to Figures 2 and 3 (not including F). Optionally, the system can be used to report to a reading center where original data can be stored and independently analyzed according to the center’s own protocols. In this case, the local software can be used for anonymization and cryptographically secured data transfer. (B) Details of data processing. Measurements for locations with multiple samples are averaged. Losses are calculated by subtraction from mean normal. For RSL, only DA sensitivities with predicted mediation by rods are included. An additional longitudinal analysis can be constructed when historic data are available.

**Legends**

**LA:** Light adapted  
**DA:** Dark adapted  
**CSL:** Cone sensitivity loss  
**RSL:** Rod sensitivity loss  
**SRL:** Normal rod sensitivity*  
**SNL:** Normal light adapted sensitivity*  
**SLAL:** Light adapted sensitivity*  
**SODA:** Dark-adapted sensitivity (*)  
**DA:** Dark-adapted sensitivity  
**CF:** DA cone mediated sensitivity*  
**N/D:** Undetermined  

* Location-specific
there can also be testing in the DA state.\textsuperscript{51} The main disadvantage of this system is that it is limited to testing the central visual field.

The advantages of the currently devised method of DA perimetry in an unmodified HFA are as follows: wide availability of the perimeter; familiarity to users in most eye clinics otherwise testing for glaucoma in the LA state with the size III target; flexibility of a projection perimeter to create custom tests; and the option to perform not only DA but also LA perimetry on the same instrument without time loss setting up the patient (and entering their identifying data) on another instrument. The disadvantages are as follows: the inability to determine rod mediation when rod sensitivities are reduced below normal cone sensitivities and to determine cone mediation in the DA state now that we are not using the two-color method; and, as in all automated perimetry, issues of free-viewing and fixation losses.

The long-term goal is to develop not only a clinically feasible and accessible method for measuring rod (and cone) function in various parts of the visual field but also to coordinate the transfer of raw data to reading centers that will collect and analyze the data. A suite of computer algorithms for the analysis of two-color DA perimetry was developed and advanced over the last three decades for the data acquired by the modified HFA, but a revised analysis is now needed. This was initiated in the present report, but an extensive normal database is required. Also needed is more experience with use of the method and analysis of results in large cohorts of IRD patients who are age-matched to the normal subjects. A future and necessary goal will be to determine intervisit variability of the new method. The lack of such data represents a major limitation of the present study. Although not the focus of this work, the method may also be useful for studies of AMD.\textsuperscript{52,53}

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**References**


