Article

Dark-Adapted Chromatic Perimetry for Measuring Rod Visual Fields in Patients with Retinitis Pigmentosa

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Purpose: Although rod photoreceptors are initially affected in retinitis pigmentosa (RP), the full-field of rod vision is not routinely characterized due to the unavailability of commercial devices detecting rod sensitivity. The purpose of this study was to quantify rod-mediated vision in the peripheral field from patients with RP using a new commercially available perimeter.

Methods: Participants had one eye dilated and dark-adapted for 45 minutes. A dark-adapted chromatic (DAC) perimeter tested 80 loci 144° horizontally and 72° vertically with cyan stimuli. The number of rod-mediated loci (RML) were analyzed based on normal cone sensitivity (method 1) and associated with full-field electroretinography (ERG) responses by Pearson's r correlation and linear regression. In a second cohort of patients with RP, RML were identified by two-color perimetry (cyan and red; method 2). The two methods for ascribing rod function were compared by Bland-Altman analysis.

Results: Method 1 RML were correlated with responses to the 0.01 cd.s/m^2 flash ($P < 0.001$), while total sensitivity to the cyan stimulus showed correlation with responses to the 3.0 cd.s/m^2 flash ($P < 0.0001$). Method 2 detected a mean of 10 additional RML compared to method 1.

Conclusions: Scotopic fields measured with the DAC detected rod sensitivity across the full visual field, even in some patients who had nondetectable rod ERGs. Two-color perimetry is warranted when sensitivity to the cyan stimulus is reduced to ≤20 dB to get a true estimation of rod function.

Translational Relevance: Many genetic forms of retinitis pigmentosa (RP) are caused by mutations in rod-specific genes. However, treatment trials for patients with RP have relied primarily on photopic (cone-mediated) tests as outcome measures because there are a limited number of available testing methods designed to evaluate rod function. Thus, efficient methods for quantifying rod-mediated vision are needed for the rapidly increasing numbers of clinical trials.

Introduction

Many genetic forms of retinitis pigmentosa (RP) are caused by mutations in rod-specific genes. However, treatment trials for patients with RP have relied primarily on photopic (cone-mediated) tests as outcome measures[1,2] because there are a limited number of available testing methods designed to evaluate rod function. Thus, efficient methods for quantifying rod-mediated vision are needed for the rapidly increasing numbers of clinical trials.

Psychophysical methods utilize the differences in rod and cone spectral sensitivity in order to isolate and quantify rod function.[3–5] Previously, devices such as scotopic fundus perimeters (microperimetry)[6–9] or modified static perimeters[10–13] have been used to advance our understanding of rod degeneration and disease progression of inherited eye disease.[6,10–21] However, fundus perimeters are restricted to testing the central field. Static perimeters require extensive custom modifications that may not be appropriate for multicenter clinical trials in order to isolate rod function. One solution is a protocol utilizing a commercial static perimeter under dark-adapted (DA) conditions, with a stimulus wavelength near

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the peak of the scotopic sensitivity function.[14] This technique documents rod mediation of sensitivity to a cyan wavelength stimulus through comparisons with the maximum theoretical sensitivity of cones at test locations throughout the visual field. However, this method does not take into account local cone viability, which masks rod sensitivity when the cones are diseased.

A second technique for quantifying rod function is through two-color perimetry where sensitivity to cyan is compared with sensitivity to a red stimulus at the same location.[13] A commercial perimeter equipped with a 7.5-log unit range of chromatic luminance, designed for scotopic static testing of the full visual field may be appropriate for multicenter clinical trials involving patients with RP. A large dynamic range of stimuli testing numerous locations throughout the visual field could be advantageous when RP is in advanced stages and the sum of rod function is insufficient to elicit a full-field electroretinography (ffERG) response.[20,22–28] Herein, we describe DA visual fields from patients with RP to determine the usefulness of a newly available, chromatic perimeter at quantifying rod function for upcoming clinical trials involving patients with RP.

Methods

Subjects

DA visual fields were obtained from 65 patients diagnosed with RP by retina specialists. Cohort 1 was comprised of 50 consecutive patients with RP (Table 1; mean age ± SD: 48 ± 14 years) that were referred to the Retina Foundation of the Southwest. Cohort 1 performed DA 505 nm (cyan) perimetry and ffERG. Cohort 2, 15 recruited patients seen previously (Table 2; 55 ± 18 years), performed DA two-color perimetry (cyan and red, 625 nm). Patients in cohort 2 were recruited because they had rod ffERG response amplitudes >3 μV,[25,29] within the past year. For comparison, 10 (36 ± 20.4 years) normally sighted controls performed DA two-color perimetry. Three of these controls (41 ± 14 years) also performed two-color perimetry under light-adapted (LA) conditions. This study was approved by the Institutional Review Board at UT Southwestern Medical Center. All participants signed an informed consent after the testing procedures were explained. This research was conducted in accordance with institutional guidelines and the Declaration of Helsinki.

Visual Function

Best-corrected visual acuity was measured with the Electronic Visual Acuity Tester (Jaeb Center for Health Research, Tampa, FL). The eye with the lower acuity, or the right eye if there was no difference between the two eyes, was dilated with eye drops (tropicamide 1% and phenylephrine 2.5%). The test eye was patched for 45 minutes to allow dark adaptation before testing. Visual fields were measured with the dark-adapted chromatic (DAC) perimeter (Medmont International Pty Ltd; Victoria, Australia).[30] A 1.72° stimulus (equivalent to the Goldman size V) was presented for 200 ms. The response time was set to 400 ms, and the interval between stimuli was fixed at 1.1 second. The maximum luminance of the cyan stimulus was 12.58 cd/m^2, and the dynamic range was approximately 75 dB. The maximum luminance of the red stimuli was 4.64 cd/m^2. Visual fields were also measured from normal controls following pupil dilation in the presence of a rod desensitizing background (1.85 cd/m^2) in order to determine the maximum sensitivity of cones at each test location.

Appropriate lenses were used in the central field as needed for correction of refractive errors and age-related accommodation loss. The test eye was aligned in the infrared viewing window and fixation was monitored throughout the examination. Patient-controlled pauses were encouraged as often as needed to prevent fatigue. The 12° grid had 80 test points that extended 144° across the temporal to nasal field and 72° between the superior and inferior field. The DAC perimeter has 164 points available for testing, but here we excluded the points separated by <12° to avoid weighting condensed points located in the central field. Far eccentric loci were tested after an automated relocation of the fixation target. The examination paused while the location of fixation target changed. The patient's eye was re-aligned in the viewing window before the test continued. Initially, head misalignment for some patients caused two infero-nasal points (−60°, −36°, and −48°, −36°) to be blocked by the nose (for example, see cyan field for patient number [P#] 12167 in Figs. 3E, 3F). After becoming aware of the issue, care was taken to instruct and confirm proper head alignment (head remains stationary and the eyes move to new target location). Nevertheless, these two loci were excluded from statistical analysis for all fields. A two-down, one-up staircase algorithm used a bracketing strategy to determine the threshold for stimulus detection at each point.

Table 1. Characteristics of Patients Tested with ffERG and Cyan DAC Perimetry

Group #	ID #	Age	Sex	Eye	BCVA	Clinical Diagnosis	Genotype
1	12012	39	M	OS	0.2	adRP	PRPH2 1068+3A−>T
1	11987	59	M	OS	0.2	adRP	Unknown
1	11992	54	F	OD	0.5	adRP	Unknown
1	4339	31	M	OD	1.5	adRP	RPGR c.2938_2959dup
1	10872	44	M	OD	0.5	adRP	IMPDH1 c.931G>A
1	12179	31	F	OS	0.2	adRP	Unknown
1	12180	21	F	OS	0.4	adRP	Unknown
1	8810	24	F	OD	0.4	RPiso	Unknown
1	12126	38	F	OD	0.1	RPiso	Unknown
1	12026	43	M	OS	0.6	RPiso	Unknown
1	7807	51	F	OS	0	RPiso	Unknown
1	9472	54	F	OS	0.1	RPiso	Unknown
1	6914[a]	16	M	OD	1.2	RPiso	Unknown
1	11349A[b]	80	M	OD	0.2	USHII	USH2A c.10073G>A/ VUS: c.821G>A
1	11402	53	F	OD	0	USHII	USH2A c.9571-2A>G c.14996C>T
1	7705	54	F	OD	0	XLRP carrier	RPGR c.2625_2626insA
1	11995	57	M	OD	0.4	sector RP	Unknown
2	9715	53	M	OS	0.1	adRP	RHO c.68C>A
2	10228	27	F	OS	0.3	adRP	PRPH2 c.610T>C; PRPF8 c.5792C>T
2	10746	35	F	OS	0.5	RPiso	Unknown
2	6982	49	F	OS	0.2	adRP	Unknown
2	10636	46	M	OS	0.8	RPiso	Unknown
2	6801	65	F	OD	0.1	RPiso	RHO c.68C>A
2	8438	25	F	OD	0.1	arRP	USH2A c.2299delG / c.10342G>A
2	11239	52	F	OD	0.8	RPiso	Unknown
2	12135	38	F	OD	0.8	USHII	Unknown
2	11612	33	M	OD	0.3	arRP	Unknown
2	12062	17	M	OS	0.1	USHII	Unknown
2	12167	52	F	OS	0.3	RPiso	Unknown
2	12248	58	M	OS	1	RPiso	Unknown
2	11956	56	M	OD	0.2	RPiso	Unknown
2	12083	66	F	OS	0.2	RPiso	Unknown
2	12085	14	M	OS	0.3	RPiso	Unknown
2	11971	39	M	OD	−0.1	arRP	Unknown
2	6705	40	F	OS	0.4	XLRP carrier	RPGR deletion 37,684,556-38,149,374bp
2	3973	42	F	OS	0.2	arRP	Unknown
3	11851	46	M	OD	0.2	RPiso	RHO c.68C>A
3	7933	51	M	OS	0.2	arRP	Unknown
3	4646	59	F	OS	1.2	adRP	PRPF8 c.6928A>G
3	6113	53	M	OS	0.8	RPiso	Unknown
3	11993	59	M	OS	0.3	arRP	Unknown
3	12061	56	F	OS	0.7	arRP	Unknown
3	5548	56	F	OS	0.3	arRP	Unknown
3	12041	64	F	OD	1.1	adRP	Unknown
3	11964	55	M	OD	0.3	RPiso	Unknown
3	11005	48	M	OS	0.2	adRP	SAG c.440G>T

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Table 1. Continued

Group #	ID #	Age	Sex	Eye	BCVA	Clinical Diagnosis	Genotype
3	7003	52	M	OS	0.4	USHII	Unknown
3	7704	25	M	OS	0.3	XLRP	RPGR c.2625_2626insA
3	12160	39	F	OS	0.9	arRP	Unknown
3	6139	66	M	OS	0.5	RPiso	Unknown

Group 1, mixed and rod responses; group 2, mixed response only; group 3, nondetectable ffERG.
[a] Tested but not included in analysis due to unreliable DAC examination.
[b] Same patient in cohort 2, but exams were from two separate visits 1 year apart.

The quality of each participant's exam was assessed by the percentage of false-positive responses. Exams with >15% false positives for controls and >20% for patients with RP were excluded[31,32]; based on this criterion, one exam (P#6914; Table 1) was excluded from analysis.

Defining Rod-Mediated Stimulus Detection

Two methods were used to determine whether rods or cones were mediating detection of the cyan stimulus. One method, comparable to that used by Jacobson et al.,[14] relied on the greater spectral sensitivity of rods than cones to a cyan (505 nm) stimulus. As shown by scotopic and photopic spectral sensitivity functions[5] (Fig. 1A), rods are most sensitive to wavelengths around 505 nm and are two to three log units more sensitive to this wavelength than are the cones. Like others have shown,[10,12,14] normal control DA sensitiv-

ity to the cyan stimulus was fairly uniform across the visual field, ranging from 42 to 57 dB (Fig. 1B). The maximum sensitivity of cones to the 505-nm stimulus was determined in the presence of a rod desensitizing background. The upper limit (UL) of cone sensitivity (mean + 2 SD) was used to demarcate rod-mediated detection of the stimulus. In other words, any sensitivity value higher than that shown in Figure 1C was rod mediated at that location since even normal cones would not detect it. Therefore, loci with DA sensitivity to cyan greater than the pointwise UL of cone sensitivity (Fig. 1C) were considered to be rod-mediated ("R"; method 1).

The second method used to determine whether rods or cones were mediating detection at each location utilized two-color (505 nm and 625 nm) perimetry. Differences in rod (circles) and cone (squares) sensitivity to these wavelengths are shown

Table 2. Characteristics of Patients Receiving Cyan and Red DAC Perimetry

ID #	Age	Sex	Eye	BCVA	Clinical Diagnosis	Genotype
10050	62	F	OS	0.4	adRP	HK1 c.2539G>A
4828	71	M	OD	0.1	adRP	KLHL7 c.458C>T
5931	63	F	OS	0.2	adRP	PRPH2 c.629C>G
8538	41	M	OS	0.1	adRP	PRPH2 c.647C>T
4920	65	F	OS	1.5	adRP	PRPH2 1068+3A>T
10277	51	M	OD	1.0	adRP	Unknown
7736	71	M	OS	0.3	adRP	Rho c.568 G>A
2807	67	M	OS	0.3	adRP	Rho c.568 G>A
4880	68	M	OS	0.0	adRP	Rho c.511C>T
22001	59	M	OD	−0.2	adRP	Rho c.68C>A
10063	26	F	OD	0.4	adRP	Rho c.68C>A
22012	26	F	OD	−0.1	adRP	Rho c.68C>A
22013	55	F	OD	0.4	adRP	Rho c.68C>A
11006	19	F	OD	0.5	RPiso	Unknown
11349B[a]	81	M	OD	0.2	USHII	USH2A c.10073G>A/821G>A

[a] Same patient in cohort 1, but exams were from two separate visits 1 year apart.
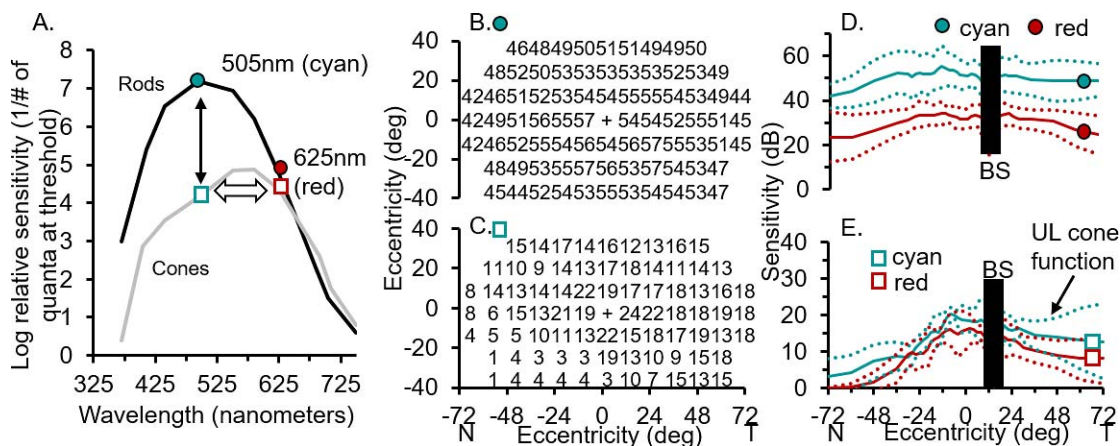
Figure 1. Rod and cone spectral sensitivity. (A) Scotopic (*top curve*) and photopic (*bottom curve*) spectral sensitivity curves. Sensitivity to 505 nm (*cyan*) and 625 nm (*red*) wavelengths differentiate rod (*circles*) and cone (*squares*) mediated detection of the stimulus. (B) DA mean sensitivity (dB) to cyan stimuli for normal controls. (C) The pointwise UL (mean −2 SD) of normal cone sensitivity to cyan. (D) The DA mean (*solid lines*) ±2 SD (*dotted lines*) sensitivity to cyan and red stimulus presented along the horizontal midlines. (E) The LA sensitivity to cyan and red stimulus. BS, blind spot. "+" marks the point of fixation and was not tested.

on the respective luminosity curves (Fig. 1A). To illustrate the principles of two-color perimetry using the DAC perimeter, sensitivity to cyan and red stimuli tested along the horizontal midline under DA and LA conditions are shown in Figures 1D and 1E, respectively. DA mean sensitivity (solid lines) ±2 SD (dotted lines) showed that normal controls were 20 to 25 dB more sensitive to cyan than to red (Fig. 1D). This is consistent with the scotopic luminosity curve where the rods are >2 log units more sensitive to 505 nm (cyan-filled circle) than to 625 nm (red-filled circle) wavelength (Fig. 1A). However, under LA conditions, the mean sensitivity to cyan was only ~5 dB greater than to the red (Fig. 1E), consistent with the cyan stimulus being 4.4 dB brighter than the red stimulus in cd/m². Thus, the spectral sensitivity difference (SSD; cyan – red) at each point stipulated whether rods were mediating detection of the stimulus (method 2). Loci were designated as rod-mediated ("r") when the SSD at a given location was >5 dB.

Full-Field Electroretinography

ERGs were obtained with the International Society for Clinical Electrophysiology of Vision (ISCEV) standard protocol.[33] Patients in the first cohort performed the ffERG on the same eye immediately after DAC cyan perimetry while the eye was DA and fully dilated. Five to 10 sweeps (responses) were computer averaged for each step (flash). Because ffERG response are not linearly distributed,[24] they were log transformed. The DA b-wave response to a 0.01 cd.s/m² flash (a rod-driven

response of ON bipolar cells) will be referred to as the *rod* response. The response was measured from the baseline before the flash to the peak of the b-wave appearing between 50 and 120 ms after the flash. Amplitudes less than 0.5 log μV (3 μV)[25,29] were considered nondetectable.

The DA 3.0 cd.s/m² flash produces a mixed response arising from photoreceptors and bipolar cells from both the rod and cone systems but is rod dominated.[33] The b-wave was measured from the trough of the a-wave to the peak of the b-wave occurring no later than 65 ms after the flash. Amplitudes less than 0.5 log μV (3 μV) were considered nondetectable.

The patients with RP were grouped according to their ffERG results as follows: group 1 patients ($n = 17$) had quantifiable response amplitudes to both the 0.01 cd.s/m² and the 3.0 cd.s/m² ffERG flashes; group 2 patients ($n = 19$) had a response to the 3.0 cd.s/m² flash but not the 0.01 cd.s/m² flash; and group 3 patients ($n = 14$) did not have measurable ffERG responses to either the 0.01 cd.s/m² or the 3.0 cd.s/m² flash. The group identity of each patient is shown in Table 1.

Statistics

The horizontal visual field axis was transposed when necessary to make all eyes right eyes for statistical analysis of pointwise sensitivity. The relationships between visual fields and ffERG responses were analyzed with least squares linear regression. One-way analysis of variance was performed to evaluate between and within group differences. Tukey-Kramer test for all pairwise
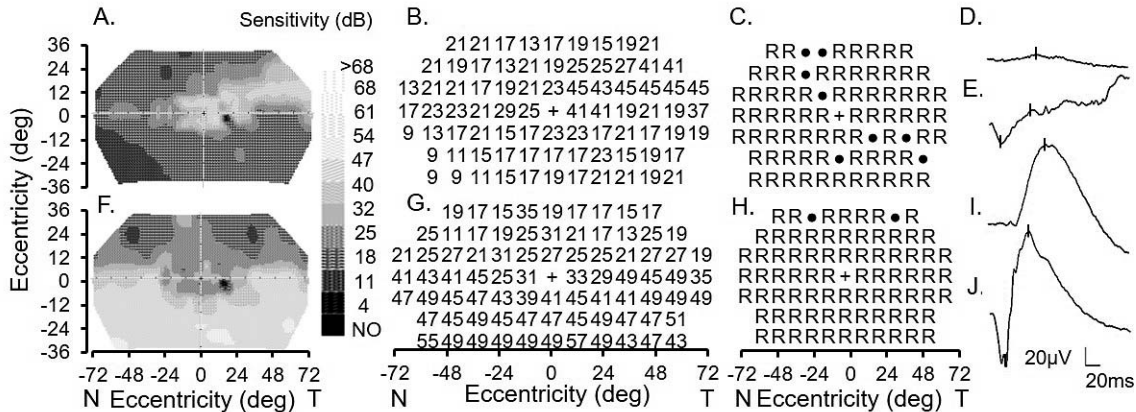
Figure 2. Patient examples from group 1 (A) P#12126 had a loss of sensitivity from the inferonasal to the superotemporal field. (B) Sensitivity (dB) was near normal (~45 dB) in the superotemporal quadrant. (C) Eight loci (•) were not detected by the rods, R. (D) The rod response to the .01 cd.s/m² flash was 0.8 log µV (6.6 µV) and the (E) mixed response to the 3.0 cd.s/m² flash was 1.6 log µV (39.7 µV). (Normal rod 72–243 µV; mixed 189–499 µV.)[36] (F) P#11995 detected all of the loci but displayed a sectoral pattern of sensitivity loss. (G) The visual field showed a sensitivity inferiorly ~45 dB. (H) There were 76 RML in the visual field. (I) The rod response to the .01 cd.s/m² flash was 2.0 log µV (95.1 µV) and the (J) mixed response to the 3.0 cd.s/m² flash was 2.2 log µV (159.1 µV).

comparisons was used to determine statistical significance. A two-tailed paired sample *t*-test was used to compare methods for ascribing rod-mediated detection of the stimulus. Data were analyzed using MedCalc Statistical Software version 16.4.3 (MedCalc Software, Ostend, Belgium).

Results

DA Cyan Visual Fields - Group 1

Patients in group 1 detected the majority of DA cyan loci throughout the field, and most patients had areas with near-normal sensitivity similar to the examples in Figure 2. P# 12126 detected all loci and had superior sensitivity ~45 dB horizontally extending from the center to 72° in the far temporal field (Fig. 2B). All but eight (70/78; 90%) loci were determined to be detected by the rods (R; Fig. 2C). The rod and mixed responses to .01 and 3.0 cd.s/m² flashes were 0.8 log µV (6.6 µV; Fig. 2D) and 1.6 log µV (39.7 µV; Fig. 2E), respectively, which were reduced compared to normal responses (rod 72–243 µV; mixed 189–499 µV).[35] P#11995 also detected all of the loci, but there was a sectoral pattern of sensitivity loss in the superior visual field (Fig. 2F). Most of the inferior field was near normal (Fig. 1B) with sensitivity ~45 dB (Fig. 2G). Ninety-seven percent (76/78 loci) of the field for P#11995 were rod-mediated (Fig. 2H). P#11995 had a normal rod response (2.0 log µV; Fig. 2I) and a slightly subnormal mixed response (2.2 log µV; Fig. 2J).

Other patterns of scotopic visual fields were evident for this group, such as superior to inferotemporal or paracentral sensitivity loss. The key features for group 1 were that the patients detected the majority of cyan loci, there were areas of near-normal sensitivity, and all had measurable responses to both the .01 cd.s/m² and 3.0 cd.s/m² ffERG flashes.

Group 2

The cyan fields for group 2 had a greater reduction in sensitivity per locus than those for group 1. All fields in this group showed a central island of vision, as well as varying degrees of paracentral sensitivity loss. P#3973 showed an incomplete paracentral scotopic scotoma extending into the temporal and inferior fields (Fig. 3A). Sensitivity in the scotoma was reduced to 5 to 15 dB (Fig. 3B), which was >30 dB lower than normal controls (Fig. 1B). The superior field had sensitivity ~35 dB, and the remaining field outside of the scotoma was ~21 to 29 dB (Fig. 3B). There were 54 (out of 78; 69%) rod-mediated loci (RML) in the scotopic field for P#3973 (Fig. 3C). The rod response to the .01 cd.s/m² ffERG was non-detectable, but the mixed response to the 3.0 cd.s/m² flash was 1.3 log µV (20.8 µV; Fig. 3D). P#12167 also had a paracentral ring scotoma in the temporal field (Fig. 3E). Scotoma loci and far peripheral loci were not detected or had sensitivity <20 dB along the edge of the scotoma (Fig. 3F). The majority of the loci in the nasal fields were ~22 dB, which was >20 dB lower than normal (Fig. 1B). Almost half of the loci (47%,
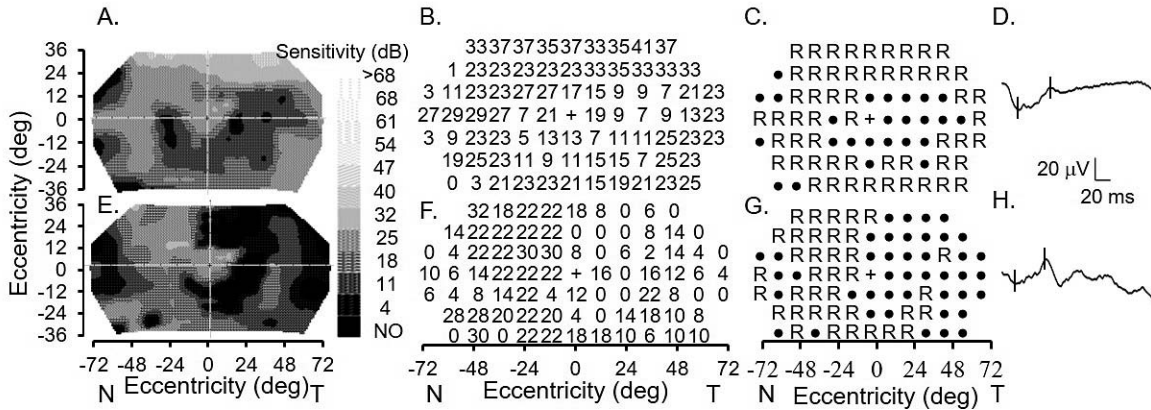
Figure 3. Group 2 patient examples. (A) P$_{\#}$3973 had an incomplete paracentral ring of decreased sensitivity from the temporal to the inferonasal field. (B) Sensitivity to the cyan stimulus was reduced throughout the field with relative sparing in the superior field. (C) P$_{\#}$3973 had 69% (54/78) loci whose detection was rod-mediated (R). (D) The mixed response (3.0 cd.s/m^2) peak-to-peak amplitude was 20.8 µV (1.3 log µV; normal 189–499 µV). (E) P$_{\#}$12167 had an incomplete temporal paracentral ring of sensitivity loss. (F) Loci in the nasal field had sensitivities of ~22 to 30 dB. (G) There were 37 (out of 78; 47%) RML primarily in the inferonasal field. (H) The mixed response amplitude was 1.4 log µV (23.1 µV).

37/78) were rod-mediated and primarily in the nasal field (Fig. 3G). The response amplitude to the 3.0 cd.s/m^2 flash was 1.4 log µV (23.1 µV; Fig. 3H). Group 2 patients retained the greatest sensitivity in the central and either the nasal or temporal field with the opposing field undetected or <10 dB. DA visual

fields for group 2 had the majority of loci reduced by more than 30 dB below normal sensitivity.

Group 3

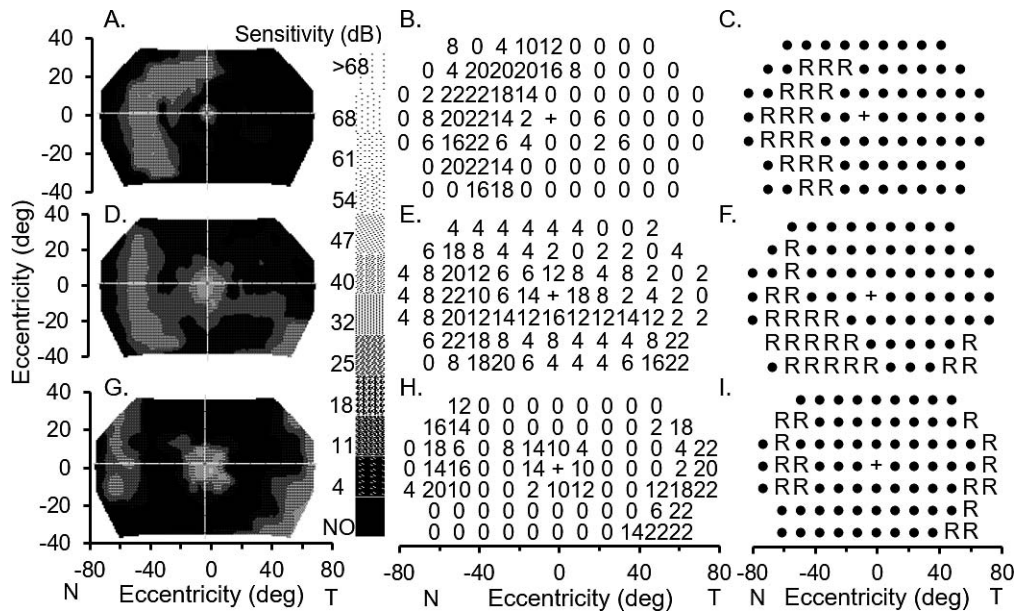P$_{\#}$6139 did not detect the stimulus in the nasal field (Fig. 4A). Most loci were either not detected (0) or

Figure 4. Group 3 patients with rod function. (A) The visual field for P$_{\#}$6139 illustrated the areas of stimulus detection. (B) P$_{\#}$6139 had reduced sensitivity (dB; 0 = not detected) in the temporal periphery and central fields. (C) There were 16 (of 78; 21%) RML ("R"), which were located in the nasal peripheral field. (D) The visual field for P$_{\#}$11005 illustrated a pattern of sensitivity loss from the superior and inferotemporal fields. (E) P$_{\#}$11005 had superotemporal loss of sensitivity. (F) Twenty-six percent (20/78) of the loci were determined to be rod-mediated. (G) The visual field map for P$_{\#}$12160 revealed an annular scotoma of the mid periphery. (H) P$_{\#}$12160 did not detect stimuli in the mid periphery and had reduced sensitivity in the central and far peripheral fields. (I) There were 15 (of 78; 19%) RML and located in the far temporal and nasal periphery.

reduced by >30 dB (Fig. 4B) compared to normal controls (Fig. 1D). There were no loci in the temporal field ascribed to rod-mediated detection (Fig. 4C). The nasal field had 16 RML, which was only 21% of all tested loci (78; Fig. 4C). P$_{\#}$11005 had a pattern of DA sensitivity loss from the superior to inferotemporal field (Fig. 4D). Sensitivities in the mid nasal and far inferotemporal periphery were ~20 dB, whereas the rest of the field was reduced to 0 to 16 dB (Fig. 4E). Twenty-six percent of all loci (20/78) were determined to be rod-mediated (Fig. 4F). P$_{\#}$12160 had a scotopic annular scotoma of the mid periphery (Fig. 4G). This patient had reduced sensitivity at central and far peripheral loci but did not detect the stimulus in the mid periphery (Fig. 4H). In the far periphery, P$_{\#}$12160 had 15 (out of 78, 19%; Fig. 4I) RML. Group 3 did not have loci with sensitivity greater than 22 dB, and most patients did not detect the stimulus throughout the majority of the field. All patients in group 3 detected the stimuli within the central 12°, but only five detected stimuli beyond the central field. Group 3 only had three patients (out of 14) with more than three RML in their DA visual field. None of the patients in group 3 had detectable ffERG responses to either the 0.01 cd.s/m^2 or the 3.0 cd.s/m^2 flash.

Rod Function by Perimetry and ffERG

Patients in group 1 ("rod") averaged 62 ± 19 RML (range 19–78 RML) in their DA visual field, which was not statistically different than controls ("ctrl"; 77.9 ± 0.4 RML; Fig. 5A). Patients in group 2 ("mixed only") had fewer RML (31 ± 17; range 6–55 RML). Group 3 ("none") had the least number of RML (4 ± 7 loci; range 0–20 loci; Fig. 5A). The numbers of RML for groups 2 and 3 were significantly different than all other groups (F-ratio 52.636, $P < 0.001$).

The mean total sensitivity to the cyan stimulus for group 1 was 2207 ± 902 dB (range 743–3518 dB) and was significantly lower that controls (3929 ± 1890 dB; Fig. 5B). The total sensitivity to the cyan stimulus for group 2 was 961 ± 473 dB (range 292–1635 dB; Fig. 5B). Patients in group 3 had total sensitivity to the cyan stimulus of 122 ± 211 dB (range 0–604 dB; Fig. 5B). Total sensitivity for all groups were significantly different from each other (control > group 1 > group 2 > group 3; $F = 77.968$; *$P < 0.001$; Fig. 5B).

These results led us to question whether the total number of RML was associated with the response amplitudes to the .01 cd.s/m^2 ffERG flash. The
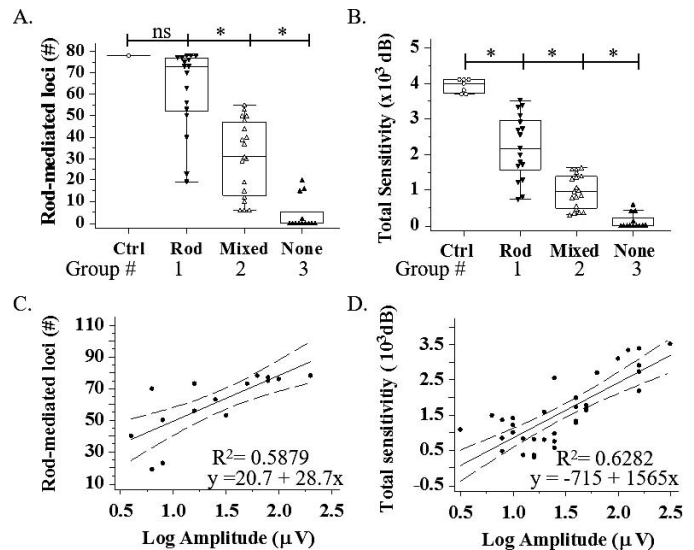

Figure 5. Analysis of DA sensitivity to the cyan stimulus and relation to ffERG. (A) The number of RML and the (B) total sensitivity for normal controls (Ctrl) and patients with RP grouped by ffERG response. ns, not significant; *$P < 0.001$; **$P < 0.0001$ (C) The number of RML had a strong positive correlation ($P < 0.0003$; $r = 0.5879$) with the log amplitude response to the .01 cd.s/m^2 ffERG flash. (D) The total sensitivity to the cyan stimulus had a strong positive correlation ($P < 0.0001$, $r = 0.7926$) to the log mixed response to the 3.0 cd.s/m^2 flash.

number of RML plotted against the patient's log rod response revealed a strong correlation ($P < 0.0003$; $r = 0.5879$) where a larger rod response was associated with a greater number of loci ascribed to rod function (Fig. 5C). These results indicated that that 59% of the response amplitude was explained by variations in the number of points detected by the rod system (Fig. 5C). Approximately 35 RML were necessary to produce a detectable rod amplitude of 0.5 log μV (3 μV) and 29 RML (95% CI: 15–42) were required to increase the rod response amplitude by 1 log unit.

The mixed response to the 3.0 cd.s/m^2 ffERG flash and the total sensitivity reflect both rod and cone activity. Therefore, the total sensitivity of the DA cyan visual fields was used for comparison to the patient's mixed response to the ffERG 3.0 cd.s/m^2 flash. There was a positive correlation between the total sensitivity and the mixed response ($P < 0.0001$; $r = 0.7926$). The total sensitivity required to raise the log amplitude to the 3.0 cd.s/m^2 flash by 1 log unit was 1565 dB (95% CI: 1145–1984), and 63% of the variation in the mixed response was attributable to the total scotopic visual field sensitivity (Fig. 5D).
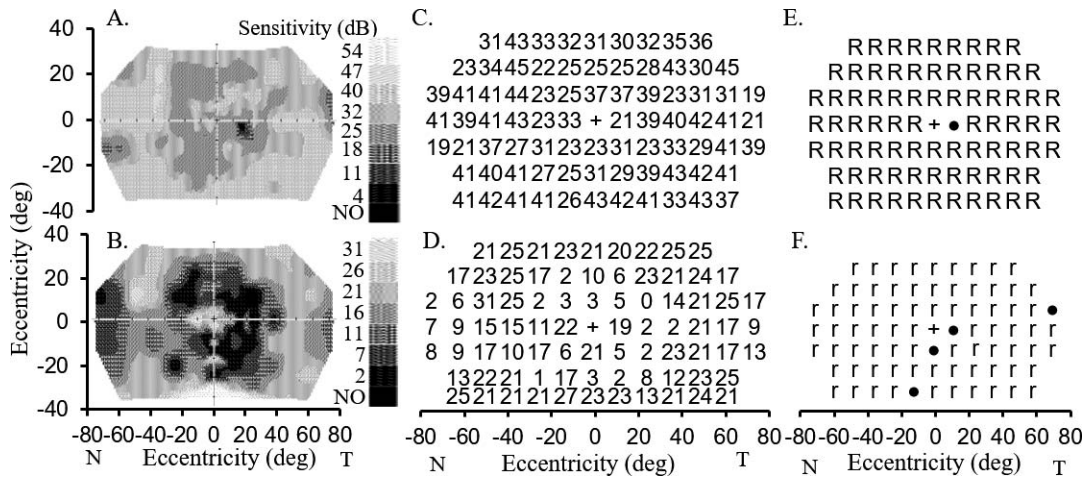
Figure 6. Verification of rod-mediated detection of spectral stimuli. P#4828 was tested with (A) cyan and (B) red stimuli. The sensitivity (dB) was near normal but reduced in a ring scotoma of the mid periphery in response to the (C) cyan and (D) the red stimulus. (E) Rod-mediated detection determined from method 1 indicated that 77 (of 78; 99%) RML comprised the field. (F) Application of method 2 confirmed that P#4828 retained rod function at 74 (95%) loci (r).

Identifying Rod Function by SSD

Method 1 assigned RML based on the cone sensitivity measured from normal controls (Fig. 1C). However, this is a conservative criterion since cone sensitivity in RP is rarely normal. Low scotopic sensitivity values may still be generated by rods in the diseased retina if the cone receptors are sufficiently insensitive. As an alternative approach to identifying the photoreceptors mediating detection, SSDs between cyan and red wavelength stimuli were evaluated from the second cohort of patients with RP (Table 2) and compared to the results using method 1.

P#4828 was tested with cyan (Fig. 6A) and red stimuli (Fig. 6B). Sensitivity was decreased in a paracentral ring in response to the cyan (Fig. 6C) and red stimuli (Fig. 6D). Rod-mediated detection assigned by method 1 indicated that 77 RML (99% of 78 RML) comprised the field (Fig. 6E). Considering SSD, method 2 found 74 RML (95%; Fig. 6F). Similarly, P#22013 also showed a paracentral ring scotoma when tested with the cyan stimulus (Fig. 7A). However, the red stimulus was only detected in the central field (Fig. 7B). Sensitivity to cyan stimuli had a gradient loss from the superonasal to the temporal
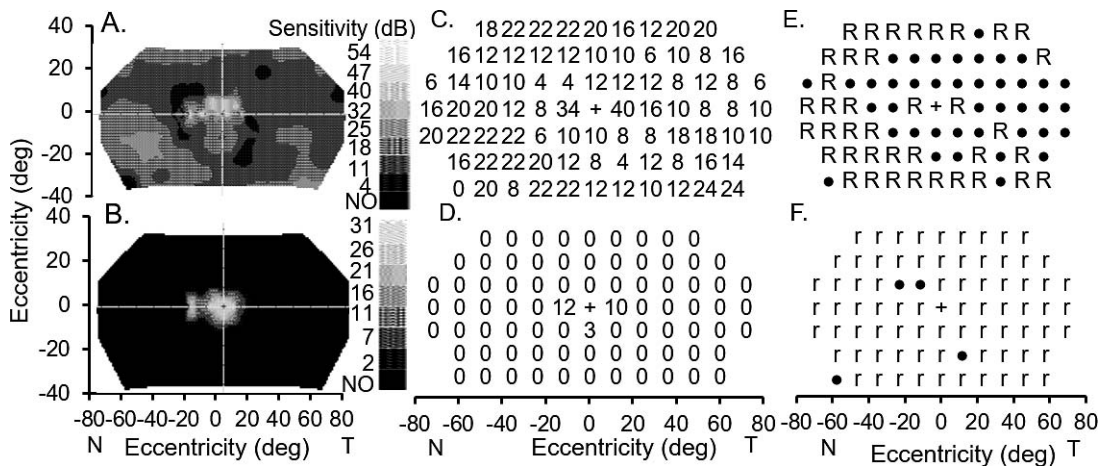

Figure 7. Verification of rod-mediated detection of spectral stimuli. (A) P#22013 was tested with cyan and (B) red stimuli. (C) The sensitivity (dB) to cyan stimulus had a pattern decrease from the superonasal to temporal field. (D) The red stimulus was seen at three central loci with reduced sensitivity. (E) Rod-mediated detection determined from method 1 indicated that 38 (of 78; 49%) RML comprised the field. (F) Application of method 2 revealed that P#22013 retained rod function at 75 (of 78; 96%) loci (r).
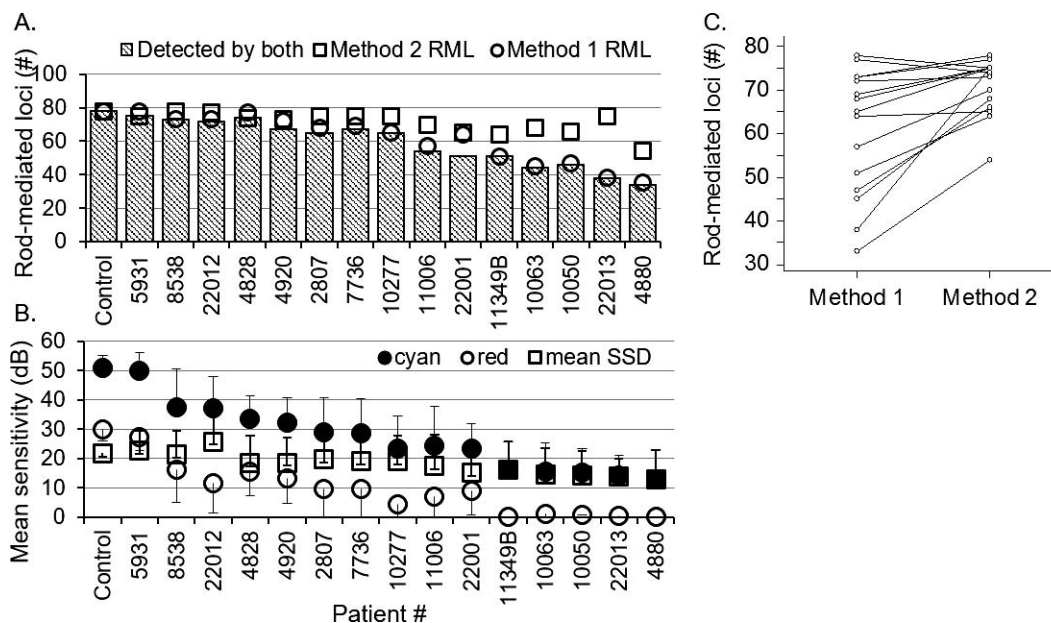
Figure 8. Comparing indices of RML. (A) The number of RML based on method 2 (*squares*), indexed by method 1 (*circles*), and identified by both methods (*gray bar*). (B) Patient mean sensitivity to the cyan (*black circles*) and red (*open circles*) stimuli. The squares are the mean SSD between the cyan and red stimuli. (C) A paired sample *t*-test between the two methods showed that assigning rod-mediation based on method 1 underestimates the number of RML especially when number of RML is low. The mean difference was 10.3 $\pm$ 11.0 loci. $P = 0.0029$.

field (Fig. 7C). The red stimulus was detected at three central loci with reduced sensitivity (Fig. 7D). Rod-mediated detection assigned by method 1 indicated that there were 38 (out of 78 loci; 49%) RML in the peripheral field (Fig. 7E). However, considering SSDs, method 2 revealed that P#22013 retained rod function throughout 96% (75 out of 78 loci) of the field (Fig. 7F).

Evaluating the number of RML by method 2 (SSD) disclosed that patients in the second cohort of patients with RP had a mean 71 $\pm$ 6 loci (range 54–78; squares; Fig. 8A). Assigning rod-mediation to loci based on method 1 determined that 61 $\pm$ 15 loci were rod-mediated (range 33–78; circles; Fig. 8A). The mean SSD (squares) in each patient was approximately 20 dB, regardless of the mean sensitivity to cyan (black circles; Fig. 8B). A paired sample *t*-test between the two methods showed that assigning rod-mediation based on method 1 did not detect as many RML as method 2 (mean difference 10.3 $\pm$ 11.0 RML; $P = 0.0029$; Fig. 8C).

Discussion

There is increasing need for a standardized device to quantify rod photoreceptor function due to the growing number of clinical trials enrolling patients

with RP. The main objective for this study was to assess DA visual field sensitivity from patients with RP utilizing a new, commercially available static perimeter. To this end, sensitivity to chromatic stimuli was measured, quantified for rod function, and related to scotopic ffERG response amplitudes.

Normal control sensitivity to the cyan stimulus (42–57 dB) was within the dynamic range of the DAC perimeter (0–75 dB). Unlike with modified perimeters, we did not need to add filters or light-attenuating goggles to assess normal controls. Nevertheless, most of our results were similar to previously published data, showing normal control sensitivity higher in the inferior field (superior retina) than in the superior field. This is consistent with a greater concentration of rhodopsin in the superior retina.[27]

DA sensitivity to the cyan stimulus was associated with scotopic ffERG responses, similar to previous studies of patients with RP.[23] Due to the strong correlation between the number of RML in the DA visual field and the response to the ffERG .01 cd.s/m² flash (Fig. 5A), we conclude that the loci indexed as being rod-mediated were indeed the result of quantifiable rod function. This is important because patients with RP have varying degrees of rod and cone dysfunction, and often the rod response to the .01

cd.s/m^2 ffERG flash is unmeasurable as with our patients in groups 2 and 3.[20,22,26] Finding RML in the peripheral fields from the three patients in group 3 (Figs. 5, 6) highlights the importance of testing the far periphery and shows that rod function can be quantified in the absence of a detectible scotopic ffERG response.

With method 1, locations were identified as rod mediated if sensitivity exceeded the UL of normal cone sensitivity. While efficient and appropriate when DA sensitivity is high, it becomes less appropriate as DA sensitivity drops in more advanced disease. Method 1 was compared with two-color perimetry (method 2) in a second cohort of patients tested because they had ERG evidence of rod function. We found that the number of RML identified through the two methods was similar when sensitivity was high. Method 2, however, identified more RML in patients with lower mean sensitivity. By comparing to the UL of normal cone sensitivity, method 1 fails to account for the fact that cone sensitivity is also decreasing over time in these patients. Method 2 takes account of this by using the difference in sensitivity to 505 nm and 625 nm stimuli to identify RMLs.

Conclusions

With the commercially available DAC, we were able to quantify rod function in patients who did not have measurable ffERG responses. Additionally, the use of scotopic full-field perimetry in RP resulted in a topographical map of rod-mediated visual field, which will be important during rod-targeted treatment trials. Most importantly, this instrument provides the capability for utilizing rod function as an outcome measure in clinical trials involving patients with RP, as well as other retinal degenerative diseases.

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