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28 **Abstract**

29 **Purpose:** Clinical assessment of rod and cone photoreceptor sensitivity often involves the use of
30 extended dark adaptation times to minimise cone involvement or the use of bright adapting
31 backgrounds to saturate rods. In this study we examine a new rod / cone sensitivity test which
32 requires minimal dark adaptation. The aim was to establish whether rod/cone sensitivity losses can
33 be measured reliably in patients with retinal diseases that selectively affect rods or cones when
34 compared to age-matched subjects with normal vision.

35
36 **Methods:** Flicker modulation thresholds (FMTs) were measured psychophysically using cone-
37 and rod-enhanced stimuli located centrally and in four quadrants at 5° retinal eccentricity in 20
38 patients (age range: 10 – 41 years) with cone-dominated (Stargardt’s disease or Macular dystrophy;
39 n = 13) and rod-dominated (Retinitis Pigmentosa; n = 7) disease. These data were compared
40 against age-matched normals tested with identical stimuli (Hathibelagal et al., 2020).

41
42 **Results:** Across all retinal locations, cone FMTs in cone-dominated diseases (Median ± IQR:
43 32.32 ± 28.15% for central location) were greater than a majority (83%; 49/59) of corresponding
44 rod FMTs (18.7 ± 3.29%; p = 0.05) and cone FMTs of controls (4.24 ± 2.00%). Similarly, rod
45 FMTs in rod-dominant disease (14.99 ± 22.58%) were greater than a majority (88%; 29/39) of the
46 corresponding cone FMTs (9.09 ± 10.33%) (p = 0.13) and rod FMT of controls (6.80 ± 2.60 %).

47
48 **Conclusions:** Cone-specific deficits were larger than rod-specific deficits in cone-dominated
49 diseases and vice versa in rod-dominated disease. These results suggest that the new method of
50 assessing photoreceptor sensitivity has potential application in detecting specific rod/cone losses
51 without the need for dark adaptation.

52
53 **Keywords:** rod, cone, temporal contrast sensitivity, Stargardt’s dystrophy, retinitis
54 pigmentosa

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56 **1. Introduction**

57 Hereditary retinal diseases can be either classified as rod - dominated (e.g., Retinitis Pigmentosa
58 and Rod-cone dystrophy) or cone - dominated diseases (e.g., Cone-rod dystrophy and Stargardt's
59 disease) based on the predominant type of photoreceptor sensitivity loss.¹ These diseases cause a
60 loss of visual function with consequences for the quality of life.² Treatment options for hereditary
61 retinal diseases have been limited but recent advances have resulted in a number of new therapies
62 that are currently in clinical trials to determine their efficacy.³⁻⁷ The need to detect changes in
63 sensitivity that fall outside normal age limits to estimate disease severity and to monitor either the
64 natural progression of the disease or the effectiveness of treatment have therefore become more
65 important.³⁻⁷ In general, any test of the visual function should be rapid, easy to execute, sensitive
66 and reliable to identify small alternations in functionality, and potentially act as clinical
67 markers/endpoints^{8,9} to monitor disease progression¹⁰⁻¹⁴ and treatment outcomes.⁶ In the context
68 of inherent retinal diseases, full field and multifocal electroretinography (ERG) is currently the
69 most commonly used objective test to measure rod and cone photoreceptor sensitivity.¹⁵⁻¹⁹ For
70 instance, multifocal ERGs can be useful in the diagnosis of local cone deficits in Stargardt's
71 disease²⁰ when compared to the more diffuse dysfunction encountered in generalized cone
72 dystrophy. However, ERG techniques do not provide information on functional vision^{15, 21} and
73 typically requires long dark adaptation times¹⁵⁻¹⁹ and the use of a bright flickering target²² which
74 can be uncomfortable for some patients. Other tests measure either cone or rod function, but not
75 both. For example, contrast sensitivity^{23, 24}, colour vision^{25, 26} and visual acuity are typical
76 measures of cone functions in central vision. Perimetry can identify changes in retinal sensitivity
77 in rod-specific diseases²⁷, but the isolation of rod and cone-specific responses are poor. While
78 such an isolation of photoreceptor function is possible with dark-adapted chromatic perimetry²⁸⁻
79 ³¹, adaptometry³²⁻³⁵ and silent substitution techniques,³⁶⁻³⁹ these procedures are tedious and
80 typically require 15 - 30 minutes of dark adaptation.²¹ They have therefore remained laboratory
81 procedures for most part and are yet to be reliably translated into a clinical setting for testing
82 patients with visual impairment.⁹

83

84 A novel psychophysical approach – the Flicker-*plus* test executed on the Advanced Vision
85 Optometric Tests (AVOT) setup – involving the measurement of monocular flicker modulation
86 thresholds (FMTs), was recently described by our group for testing of rod and cone-mediated

87 vision with minimal adaptation time.⁴⁰ FMTs describe the smallest modulation thresholds at the
88 corresponding temporal frequency employed in the test needed to detect rapid flicker on 71% of
89 presentations. The stimulus causes no change in time-averaged retinal illuminance and the
90 modulation depth is quantified using Michelson contrast. The stimuli for evaluating the
91 functionality of the two types of photoreceptors in this test is based on exploiting the well-known
92 differences in rod and cone sensitivities to different spatiotemporal properties such as temporal
93 frequency, retinal illuminance, size, duration and spectral composition.⁴⁰ Normative data of
94 rod/cone FMTs across a wide age range were also described in that study.⁴⁰ Central and parafoveal
95 (5°) rod and cone-enhanced FMT remained invariant up to 45 years of age, however beyond that
96 age, both rod and cone FMT increase at a faster rate with increasing age and more specifically rod
97 FMTs increased at a faster rate than cone FMT.⁴⁰ Interestingly, there was no difference in cone
98 and rod FMTs across the four parafoveal locations (superonasal, superotemporal, inferonasal and
99 inferotemporal).⁴⁰ Values higher than the upper limits of this normative database may signal
100 deficits in flicker processing of subjects and could potentially be used to identify patients with
101 cone and rod photoreceptor disease. The present study evaluates the capability of the Flicker-*plus*
102 test in identifying selective deficits of cone and rod-photoreceptor functions in patients with the
103 aforementioned cone-dominant and rod-dominant diseases. This study tests the following two
104 complementary hypotheses related to cone and rod FMTs in these patients: 1) Cone FMTs in
105 patients with cone-dominated diseases will be significantly higher than the corresponding rod
106 FMTs and higher than the upper limit of cone FMT's of age-matched controls; rod FMTs of these
107 patients may not be significantly different from that of age-matched controls. 2) Rod FMTs in
108 patients with rod-dominated diseases will be higher than the corresponding cone FMTs and higher
109 than the upper limit of rod FMT's of age-matched controls; cone FMTs of these patients may not
110 be significantly different from that of age-matched controls.

111

112 **2. Methods**

113 Twenty patients with rod- or cone photoreceptor-dominated disease participated in this study.
114 These subjects were recruited from the outpatient department of the Vitreo-retinal services of the
115 L V Prasad Eye Institute (LVPEI), Hyderabad, India. The protocol and ethics for the study were
116 approved by the Institutional Review Board at the LVPEI, Hyderabad, India. All the procedures
117 in the study were conducted in accordance with the tenets of the Declaration of Helsinki. Written

118 informed consent was obtained from all the participants before they took part in the study. The
119 written consent was provided by the parents or the local guardian for participants aged <18 years.
120 Participants who are diagnosed as having Retinitis Pigmentosa (rod-dominated; n = 7; 5 males
121 and 2 females; Mean \pm 1SD age: 32.4 \pm 13.5yrs) and or Stargardt's disease/macular dystrophy
122 (cone-dominated; n =13; 8 males and 5 females; Mean \pm 1SD age: 23.3 \pm 12.2yrs) were included
123 in the study. The diagnosis was confirmed by retina specialists, if at least one of the three following
124 criteria were met: 1) Presence of retinal flecks or Bulls' Eye maculopathy for cone-dominated
125 disease and presence of arteriolar attenuation and bony spicules appearance for rod – dominated
126 disease during the clinical presentation; 2) Fundus autofluorescence (FA) revealing a peripheral
127 ring of hyperfluorescence spots around the central macular region of hypofluorescence confirming
128 the presence of Stargardt's disease⁴¹; 3) Full-Field electroretinography responses showing
129 impaired rod or cone-specific responses. None of the patients had any systemic syndrome
130 associated with the ocular pathology. Only participants aged \geq 10 years were recruited as a pilot
131 study in our lab found that older participants were more reliable and consistent in their test
132 responses when compared to their younger counterparts.

133

134 The Advanced Vision Optometric Tests (AVOT) is commercially available equipment developed
135 at the City, University of London^{40, 42} that supports a number of psychophysical assessments of
136 visual functions. The AVOT software runs on a laptop computer with Windows operating system.
137 The user interface is displayed on the laptop monitor while the visual stimuli are displayed on a
138 second monitor that is fully calibrated for luminance and chromaticity. In the experimental set up
139 available at LVPEI, the stimulus monitor is a 24" calibrated visual display (EIZO, Model
140 ColorEdge CS2420; EIZO Corporation, Japan) that is separated from the laptop display by a black
141 curtain, such that the patient can only see the stimulus monitor without any stray light from the
142 latter. The calibration of the display was performed using a photometer (Mavo-Monitor USB,
143 Gossen, Germany) and custom-built program (LUMCAL; City Occupational, Ltd., London, UK).
144 The stimulus is controlled by the experimenter using the Flicker-*plus* module, which runs on the
145 laptop. The room light was turned off while the test was carried out.

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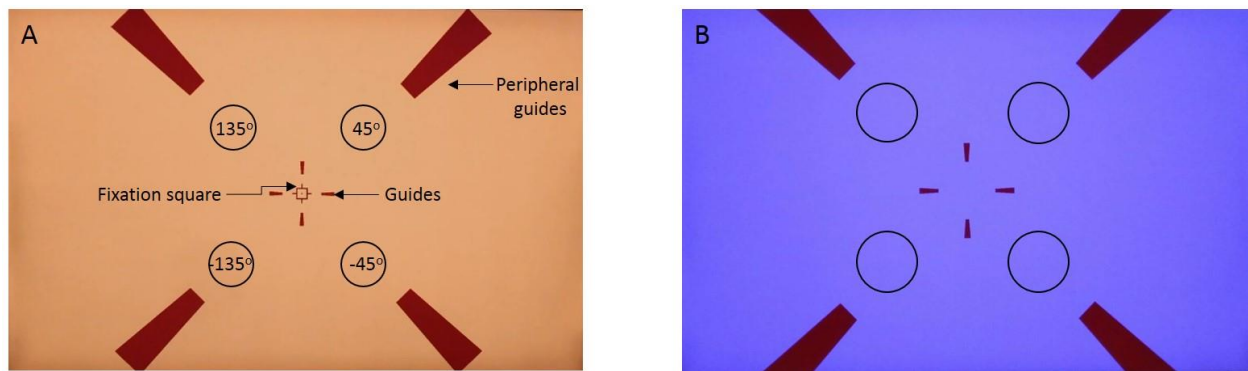
147 For assessing cone thresholds, the central (0°) test stimulus was 30' in angular subtense and the
148 four parafoveal test stimuli at 5° eccentricity were 60' each, all at 1m viewing distance. The

149 photopic luminance of the display was 24 cd/m^2 . The CIE chromaticity co-ordinates,
150 scotopic/photopic (S/P) ratio, temporal frequency and presentation duration were (0.58, 0.32), 0.9,
151 14.9Hz and 334ms, respectively. For assessment of rod thresholds, the central stimulus subtended
152 $45'$ while the four parafoveal stimuli subtended $90'$, all at 1m viewing distance. The CIE
153 chromaticity co-ordinates, S/P ratio, temporal frequency and presentation duration for rod-
154 enhanced stimuli were (0.18, 0.077), 9.0, 5Hz and 600ms, respectively. As part of a related study
155 carried out during the development of the test, different stimulus sizes have been investigated. The
156 improvement in rod threshold for stimulus sizes greater than $45'$ in central vision was minimal.
157 We wanted to ensure that the stimulation of the retina was restricted to small regions and to avoid
158 the averaging of responses, in patients with localized changes in sensitivity. Even with the $45'$ size,
159 the area stimulated may be significantly larger as a result of micro fluctuations in fixation during
160 the stimulus. Although the stimulus presentation time during the Flicker-plus test is only 600 ms
161 for the rod condition, the fluctuation in eye movement while attempting fixation can be as large as
162 $30 - 45 \text{ min of arc}^{43}$. It is therefore reasonable to expect that a region of $\sim 90 \text{ min arc}$ may be
163 stimulated with a stimulus diameter of 45 min of arc . The photopic luminance of the uniform
164 background was 0.5 cd/m^2 and this was achieved by the subjects' wearing spectrally calibrated
165 neutral density filters. The temporal profile of the stimulus was sinusoidal with equal time-
166 averaged luminance and it was the same for both rod and cone stimuli. The calibration of the
167 display was also adjusted automatically by the program to take into account the spectral
168 transmittance characteristics of the filters. For both stimuli, the background and the target always
169 had the same spectral composition to eliminate potential inaccuracies in contrast computations
170 caused by spectrally selective, pre-receptor filters in the eye.⁴⁴ The order of the rod and cone
171 tests was randomized. Adaptation times of $\sim 15\text{s}$ for cones and 90s for rods were employed in all
172 tests. Preliminary tests revealed that the use of natural pupil size and extended dark adaptation
173 times of up to 15minutes does not cause significant changes in the measured thresholds. The
174 rod/cone flicker test is not intended to provide full isolation of each class of photoreceptors but to
175 produce large differences in sensitivity between the two major photoreceptor classes (rod and
176 cone). Therefore, cone (S and M cone) intrusions may still be present.

177

178 Only participants who had visual acuity of at least 20/200 (logMAR 1.0) or better with spectacle
179 correction were recruited for this study to ensure adequate fixation stability on a well-defined

180 fixation target located in the centre of the display and flanked by diagonal peripheral guides, all
181 pointing towards the centre of the display. In addition, a square target imaged at the centre of the
182 screen preceded each stimulus presentation. The combination of guides and the briefly presented
183 fixation target made it easier for the subject to keep his / her point of regard on the centre of the
184 screen during each stimulus (Figure 1). Each presentation was followed by an auditory beep. The
185 tests were carried out monocularly and only eyes which met the inclusion criteria were tested.
186 Based on the previous pilot study in healthy controls, the repeatability of FMT measurements was
187 estimated to be ~2%.



188
189 **Figure 1. Schematic of the cone (left panel) and rod-enhanced (right) test conditions used for the Flicker-**
190 **plus test.** The numbers in panel A indicate the position in degrees, where the stimulus would appear in one
191 of the parafoveal locations. ($\pm 45^\circ$ and $\pm 135^\circ$). The central stimulus (0°) is not shown in the figure.
192 However, it would appear on the place where fixation square is shown (panel A). There are also central
193 and peripheral guides to aid fixation. Note that the actual size of the stimulus is not shown in the figure, it
194 is only for representation and also the original stimulus does not have any outline.

195
196 The stimulus was presented either centrally or in one of the four quadrants (45° - Upper Right;
197 135° - Upper Left; -135° - Lower Left and -45° - Lower Right). The participant's task was to
198 indicate the location of the stimulus by pressing raised buttons on a numeric keypad, which
199 mirrored the five test locations. Participants were instructed to press the sixth button, if they were
200 unable to locate the target, in which case, the program randomly assigned the response to one of
201 the five locations. In instances (25%, 5/20 participants), where the participant was unable to use
202 the keypad, the examiner pressed the appropriate key, based on the participant's verbal response.
203 FMTs are measured at each of the five locations in the visual field using five randomly interleaved
204 2-down 1-up adaptive staircases wherein the step size varied commensurate with the subject's
205 response to arrive at the threshold quickly. The staircases terminated at 9 reversals each and the
206 threshold was taken as the average of the last 6 reversals of each staircase.

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Data analysis was carried out using SPSS software (IBM SPSS, version 25; IBM Corp., Armonk, NY, USA). The figures were created using ggplot2 package built in R 3.6.3 (<http://www.r-project.org/>) under R studio 1.2.5001 (RStudio, Boston, MA, USA) and SPSS. The data was not normally distributed as tested by Shapiro Wilk test ($p < 0.05$). Therefore, non-parametric tests were used for comparison between flicker modulation thresholds for rod and cone-dominated diseases. The rod and cone FMT in patients with inherited retinal diseases will be compared against the age-matched database.⁴⁰

3. Results

Twenty-two subjects that passed the inclusion criteria were recruited for the study. Amongst them, two participants were unable to complete the learning mode and were not included in the main study. Therefore, a total of 20 subjects finally participated in the study - the testability rate of the Flicker-*plus* test for the current study was therefore ~91% (20/22). The mean ($\pm 1SD$) age of the participants was 25 ± 12 years. There were ten subjects in whom both eyes were tested, only one eye from each subject was randomly included for analysis. Randomization was achieved by applying the formula “RANDBETWEEN (0, 1)” formula in Microsoft Excel (2013). In instances when rows corresponding to participants were assigned “0” (zero), the right eye was selected and in case of “1” (one) the left eye was chosen. Mean of two eyes in the same subject can be obtained when intraclass correlation between the two eyes is close to 1⁴⁵. However, in the current dataset, only two of the 10 participants had an intraclass correlation close to 1 (≥ 0.90). Therefore, the mean response was not utilized to keep it consistent across all subjects. Twenty eyes of 20 subjects (7 females, 13 males) were included for the final analysis. A sub-analysis involved estimation of the Coefficient of Repeatability (CoR) to compare the differences in the independent measures of rod and cone FMTs between the two eyes of the same subject. The mean ($\pm SD$) CoR across the subjects was 7.89 % (± 4.59 %). Table 1 shows the clinical characteristics of patients who met the inclusion criteria and were recruited for the study. In general, the time taken for completion of the test ranged between 10-15 minutes for each condition.

Table 1: Clinical characteristics of the patients recruited in the study

	Age, (years)	Sex	BCVA (logMAR)		Fundus findings	ERG/FA findings
			OD	OS	Both eyes	Both eyes
1	41	F	0.70 (6/30)	0.50 (6/19)	Pigmentary changes, arteriolar attenuation	Rod: Absent; cone: almost extinguished; left eye less affected
2	35	F	0.10 (6/7.5)	0.10 (6/7.5)	Bony spicule pigmentation, attenuated arteries and veins	Rod: Absent; Cone: slightly present
3	39	M	0.0 (6/6)	0.0 (6/6)	Arteriolar attenuation pigmentary changes	-
4	10	M	0.40 (6/15)	0.40 (6/15)	RPE changes all over with attenuated arteries	Both responses are extinguished
5	39	M	0.10 (6/7.5)	0.10 (6/7.5)	Bony spicules, Arteriolar attenuation, disc pallor+, Stable	-
6	17	M	0.10 (6/7.5)	0.20 (6/9.5)	Arteriolar attenuation pigmentary retinal degeneration	-
7	40	M	0.10 (6/7.5)	0.10 (6/7.5)	Bony spicules, Arteriolar attenuation, disc pallor+, Stable	-
8	37	F	0.90 (6/48)	0.90 (6/48)	Macular scar	-
9	36	M	0.20 (6/9.5)	0.80 (6/38)		Auto hypofluorescence with surrounding hyper autofluorescence
10	13	F	1.00 (6/60)	1.00 (6/60)	RPE changes and Flecks	-
11	21	M	0.90 (6/48)	0.90 (6/48)	RPE Atrophic patch	-
12	48	M	0.60 (6/24)	0.20 (6/9.5)	Arteriolar attenuation, dystrophic patch	Auto hypofluorescence with surrounding hyper autofluorescence
13	11	M	1.20 (6/95)	1.00 (6/60)	Mild attenuation; RPE hypo and hyper pigmentary changes	Auto hypofluorescence with surrounding hyper autofluorescence
14	30	F	0.90 (6/48)	0.90 (6/48)	Multiple whitish yellow spots +	Auto hypofluorescence with surrounding hyper autofluorescence
15	30	M	0.80 (6/38)	0.60 (6/24)	RPE changes and Flecks	-
16	12	F	0.80 (6/38)	0.80 (6/38)	Fundus normal	-
17	11	F	0.70 (6/30)	0.90 (6/48)	Macular degeneration patch	-
18	10	M	0.70 (6/30)	0.80 (6/38)	Macular degeneration patch	Auto hypofluorescence with surrounding hyper autofluorescence
19	27	M	0.90 (6/48)	0.90 (6/48)	Bull's eye maculopathy	Auto hypofluorescence with surrounding hyper autofluorescence
20	12	M	0.60 (6/24)	0.60 (6/24)	Fundus normal	Auto hypofluorescence with surrounding hyper autofluorescence

240

241 Table 2 shows the median [interquartile range (IQR)] cone and rod FMTs obtained in cone- and
 242 rod-dominated disease along with the age-matched values of controls from Hathibelagal et al
 243 (2020).⁴⁰ Ninety-nine percent (185/187) of the central and parafoveal cone and rod photoreceptor
 244 FMTs in the patients with cone- and rod-dominated diseases were higher than the corresponding

245 median values of age-matched controls, irrespective of the disease type (Table 2). Mann Whitney
 246 U-test revealed borderline significant differences between central cone FMTs [Median: 32.32%
 247 (IQR: 28.15%)] and the corresponding rod-FMTs [18.7 %, (3.3%); $p = 0.05$] in the cone-dominated
 248 disease (Figure 2). None of the comparisons in the parafoveal test locations were significantly
 249 different from each other ($p > 0.05$), although there was a qualitative trend for the cone FMTs to
 250 be larger than the corresponding rod FMTs (Figure 2A). None of the rod FMTs were significantly
 251 different when compared to the corresponding cone FMTs in rod-dominated diseases ($p > 0.05$).
 252 However, the qualitative trend of higher rod FMTs in comparison to cone FMTs in rod-dominated
 253 disease can be noticed in the Figure 2B.

254

255 **Table 2:** Comparison of median (IQR) central and parafoveal cone vs rod FMT in the cone and rod-
 256 dominated diseases against the normative database⁴⁰. IN, IT, ST and SN correspond to inferonasal,
 257 inferotemporal, superotemporal and superonasal parafoveal locations.

Stimuli	Cone-dominated disease					Rod-dominated disease				
	Central FMT (%)	Parafoveal FMT (%)				Central FMT (%)	Parafoveal FMT (%)			
		IN	IT	ST	SN		IN	IT	ST	SN
Cone FMT	32.3 (28.2)	13.6 (28.4)	15.4 (30.2)	29.3 (28.0)	26.8 (31.3)	9.1 (10.3)	12.8 (20.2)	13.7 (7.9)	9.6 (8.0)	12.0 (6.0)
Rod FMT	18.7 (3.3)	10.3 (5.7)	10.9 (3.6)	13.0 (4.8)	14.9 (13.1)	15.0 (22.6)	16.7 (22.6)	13.6 (26.3)	14.5 (19.5)	19.1 (9.3)
Normative database Cone FMT ⁴⁰	4.2 (2.0)	4.4 (1.5)				4.2 (2.0)	4.4 (1.5)			
Normative database Rod FMT ⁴⁰	6.8 (2.6)	5.5 (1.3)				6.8 (2.6)	5.5 (1.3)			

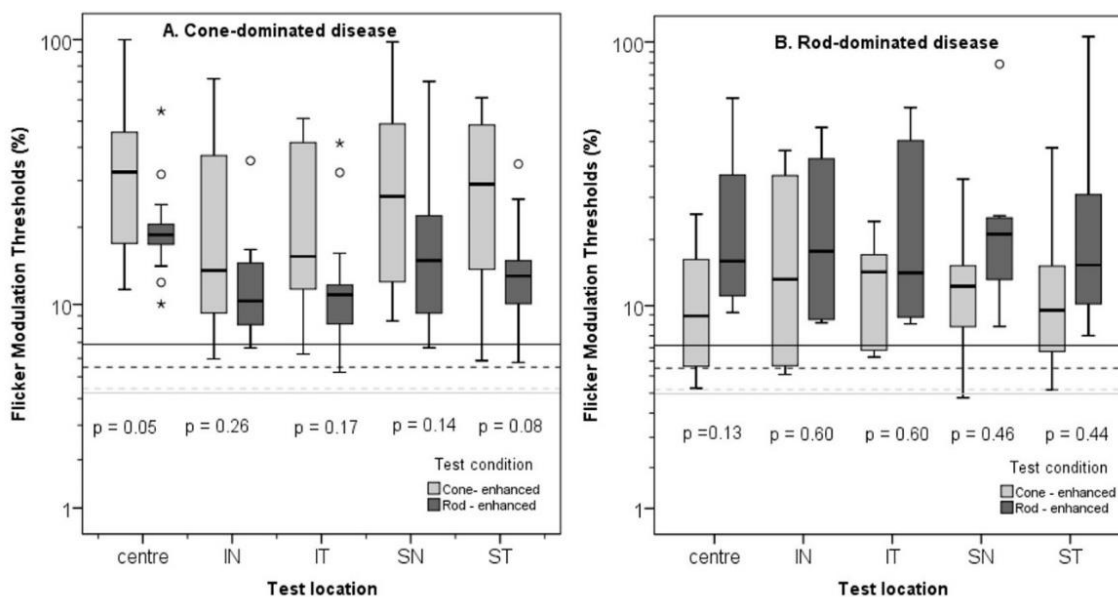
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259 Even while the median values did not reveal statistically significant differences in the cone and
 260 rod-FMT's in the two disease types, the ratio of cone to rod FMTs revealed photoreceptor-specific
 261 disease patterns (Figure 3). Cone/rod FMT ratio of >1.0 indicates that the deficits in cone
 262 photoreceptors were relatively more than those in rods and a ratio of < 1.0 indicates the reverse.
 263 Eighty-three percent (49/59) of the test locations showed cone/rod FMT ratios > 1.0 in cone-
 264 dominant diseases (Figure 3A) and 74.6% (44/59) of the test locations had cone/rod FMT ratio
 265 between 1.0 and 4.0. Only one individual exhibited cone/rod FMT ratio greater 4.0 in at least 4 of
 266 the testing locations. Eighty-eight percent (29/33) of the test conditions showed these ratios to be
 267 < 1.0 in the rod-dominant disease (Figure 3B), while 48.4% (16/33) and 39.4% (13/33) of the test
 268 locations had cone/rod FMT ratio between 0.5 - 1.0 and ≤ 0.5 respectively. These ratios were in

269 the expected direction of photoreceptor functionality loss depending on the dominance of the
270 disease type.

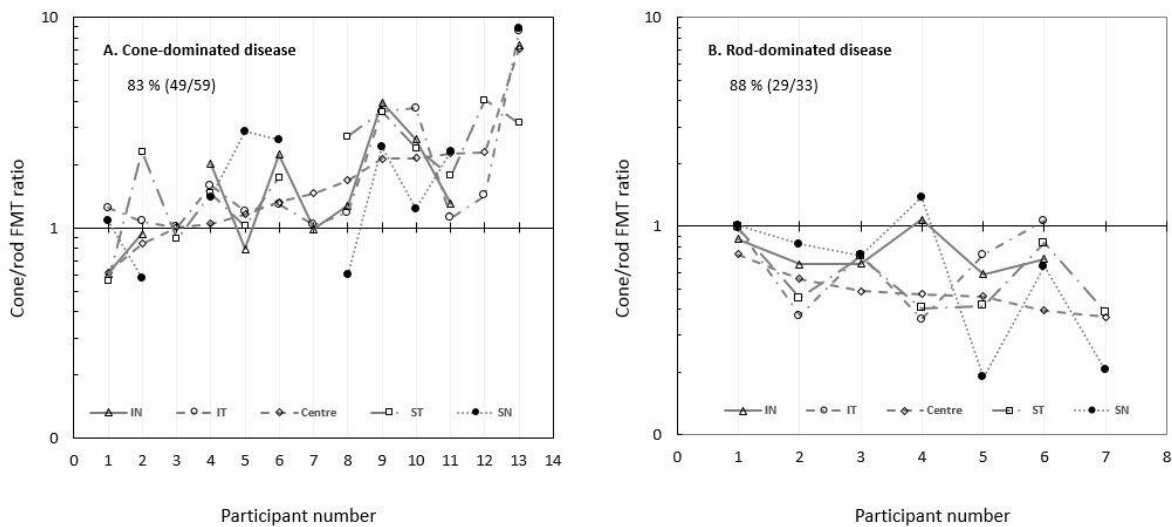
271
272 A correlation analysis was carried out to ascertain if there was any relationship between central
273 rod/cone FMTs and visual acuity. The central cone and rod FMTs were poorly and statistically
274 insignificantly correlated with the high-contrast logMAR acuity of patients in cone-dominated
275 (cone: $r = 0.37$; $p = 0.21$; rod: $r = -0.21$; $p = 0.50$) and rod-dominant (cone: $r = 0.40$; $p = 0.36$; rod:
276 $r = 0.19$; $p = 0.69$) disease.

277



278
279 **Figure 2.** Box and whisker plots of cone and rod FMTs obtained from the central and parafoveal (IN, IT,
280 SN, ST) positions for cone- (panel A) and rod-dominated disease (panel B). The thick horizontal line in each
281 box plot indicates the median, the upper and lower end of the box indicates the interquartile range, the open
282 circles represent the outliers and asterisk shows the extreme values. The solid and dashed horizontal lines
283 refer to the central (gray for cone and black for rod) and parafoveal average age-matched flicker threshold
284 values, respectively, from Hathibelagal et al (2020)⁴⁰. P-values indicate the comparison between two tests
285 conditions at each of the stimulus location in both the diseases.

286



287
 288 **Figure 3.** Ratio of cone to rod FMT in cone-dominated (panel A) and rod-dominated (panel B) diseases
 289 plotted for each subject that participated in this study. The solid horizontal lines in each of the panels at
 290 1.0 indicate that rod and cone FMTs were equal. Ratios >1 indicates cone FMT were greater than rod
 291 thresholds, indicating cone dysfunction and ratio <1 indicates rod FMT were higher than cones, indicating
 292 rod dysfunction. The different symbols indicate the five test locations namely centre (C), inferonasal (IN),
 293 inferotemporal (IT), superonasal (SN), and superotemporal (ST) quadrants. The number in panel A
 294 indicates the percentage of test locations with cone/rod FMT ratio > 1 and number in panel B indicate the
 295 percentage of test locations with cone/rod FMT ratio < 1 .

296

297 4. Discussion

298 This study evaluated a new Flicker-*plus* test designed to measure cone- and rod-mediated
 299 flicker sensitivity in patients with either cone- or rod-dominant diseases of the retina. The results
 300 reveal two principal findings. First, irrespective of the disease type (cone- or rod-dominated
 301 disease), both the rod and cone thresholds were higher than the corresponding, age- and ethnicity-
 302 matched normative values reported earlier⁴⁰ (Table 2). Second, cone FMTs were greater than rod
 303 FMTs in cone-dominated disease and the effect reversed in rod-dominated disease (Figures 2 and
 304 3). The results from this study also confirm earlier findings which show generalized flicker deficits
 305 in patients with inherited retinal degenerations.^{46, 47} More specifically, flicker deficits have been
 306 reported in patients with Stargardt's disease at all temporal frequencies (up to 50Hz) except for an
 307 intermediate range of frequencies (~5-15Hz).^{46, 48} Loss of sensitivity at high temporal frequencies
 308 have been reported in patients with retinitis pigmentosa.^{46, 47} The larger cone FMTs relative to rods
 309 (Figure 2) measured in this study and cone/rod FMT ratios above unity in cone-dominated disease

310 (Figure 3A) are consistent with previously reported studies, which also show greater cone losses
311 relative to rods in patients with cone dystrophy.^{17, 19, 23} Larger rod FMTs relative to cones (Figure
312 2) and below unity cone/rod FMT ratios in rod-dominated disease diseases (Figure 3) are also in
313 line with reports from previous studies.^{18, 49} Smaller response amplitudes in rod-specific
314 electroretinogram signals and accelerated loss in rod function when compared to cone responses
315 have been reported in patients with the rod-dominated disease such as Retinitis Pigmentosa.^{15, 50}
316 In general, flicker sensitivity losses in retinal degenerations have been attributed to loss of quantum
317 catching ability in the photoreceptors due to low photopigment density or the change in temporal
318 properties of rods and cones in response to flickering stimuli.⁴⁶

319

320 The observation of both the rod and cone FMTs being poorer than age-matched controls,
321 irrespective of disease type, indicates the absence of normal function in all photoreceptors, even
322 when clinically the disease is labelled as either rod- or cone-photoreceptor specific. Rod deficits
323 have been shown to be present in cone-dominated diseases such as progressive cone dystrophy¹⁹
324 and Stargardt's disease.¹⁴ Histopathological studies in some patients with cone dystrophy have
325 shown abnormalities in rod morphology such as the rod outer segment enlargement⁵¹, which may
326 adversely affect rod photoreceptor function, even in a cone-dominated disease. Changes that may
327 occur in proteins acting at the rod photoreceptor segments⁵² may lead to rod dysfunction in the
328 cone dystrophies. Analogously, longer dark adaptation times for rods in patients with Stargardt's
329 disease points towards rod dysfunction, potentially attributed to the accumulation of lipofuscin in
330 the retinal pigment epithelium (RPE) layer that may interfere with the visual pigment regeneration
331 process.⁵³ The presence of cone deficits in rod-dominant disease such as Retinitis Pigmentosa may
332 arise from cone cell death in this disease, perhaps due to increased oxidative stress or release of
333 rod-derived toxins or microglial activity.⁵⁴

334

335 The lack of significant correlation between high contrast visual acuity and cone/rod FMT
336 ratio is not surprising as it has been well established previously that high contrast visual acuity
337 fails to reflect early-stage photoreceptor loss in patients with inherited retinal diseases⁵⁵ and, more
338 particularly, rod FMTs. This is consistent with a previous study that showed no significant
339 relationship between FMTs and visual acuity in normal subjects⁵⁶. However, the same group also
340 showed that there is a significant relationship between FMT and visual acuity in patients with

341 macular pathology such as age-related macular degeneration.⁵⁷ The differences between the results
342 of the present study and previous findings could be attributed to differences in the disease cohort
343 (inherited retinal diseases in the present study versus age related macular degeneration in the study
344 by Brussee et al (2018)⁵⁷), younger age group (Average age: 26.8 ± 13.4 years (present study)
345 versus 77 years⁵⁷) and the flicker frequency (5 & 15 Hz (present study) versus 8 Hz⁵⁷).

346

347 Inherited retinal diseases typically have bilateral presentation⁵⁸ and it is therefore expected
348 that the FMTs will be elevated in both eyes of the patient, relative to age-matched controls. One
349 may also be tempted to interpret the difference in FMT between the two eyes of the same subject
350 as a measure of test “repeatability”. Such an interpretation is based on the assumption that the
351 disease severity between the two eyes are similar. Large inter-eye variability in FMTs were
352 observed in this study, may well be indicative of varying levels of disease severity in the two eyes
353 and location-specific differences in disease pattern between the eyes. Therefore, caution must be
354 exercised before such an interpretation is made.

355

356 Overall, this study demonstrates that with the appropriate choice of light level and spectral
357 and spatiotemporal parameters, it is possible to measure rod- and cone-specific thresholds without
358 the need for either high retinal illuminance levels or full dark adaptation. The cone/rod FMT ratio
359 metric would be useful in differentially identifying cone versus rod dominant disease types in a
360 clinical scenario, when there is uncertainty in the diagnosis. The Flicker-*Plus* test is easy to carry
361 out and has the additional advantage of measuring rod and cone-specific sensitivity at five discrete
362 locations in central vision using small stimuli. This test is not intended to replace the existing
363 diagnostic technology such as electroretinography, but to add further value that would aid
364 improved diagnosis, management, follow-up and the overall understanding of disease
365 pathophysiology. As is often the case in a challenging diagnosis, multiple investigations need to
366 be carried out by specialized personnel to confirm the presence/absence of a disease, which include
367 objective techniques such as ERG combined with psychophysical tests. Genetic testing for
368 genotyping and/or para-neoplastic panels for anti-retinal autoantibodies can also provide further
369 diagnostic value. The shared inter-professional collaboration can therefore to better diagnosis and
370 management of the disease.

371

372 The new test has some obvious limitations. First, the protocol employed measured
373 peripheral FMTs in each of the four quadrants, but only a single retinal eccentricity of 5°. This
374 choice of eccentricity was to ensure that the protocol remained consistent with age-matched
375 normative data. Changes in the FMTs at further eccentricities remain unknown, but can be
376 explored, if required. The staircase procedures employed require a minimum of five stimulus
377 locations, but both the eccentricity and the number of peripheral locations tested can be altered.
378 Although more eccentricities can be tested in the same run, the time required to complete the test
379 using randomly interleaved staircases is directly proportional to the number of stimulus locations
380 involved. Changes in the FMTs at further eccentricities remain unknown, but can be explored, if
381 required. The test is likely to be of value for use in the clinic, but further research is needed to
382 investigate the optimum number of retinal locations and eccentricities to be investigated in relation
383 to the time needed to complete the test. Second, the presence of eccentric fixation in the patients
384 that participated in this study was not tested using techniques such as scanning laser
385 ophthalmoscope⁵⁹. However, none of the participants were noted to have any obvious eccentric
386 fixation in their clinical records. This was also supported by the lack of any abnormal head posture
387 while fixating on the center of the screen. Therefore, any impact of eccentric fixation on the FMTs
388 reported here are likely to be negligible. However, those subjects who may have experienced small
389 eccentric fixation, we expect that the strong fixation stimulus and guides minimized potential drifts
390 in fixation during the stimulus. The third limitation is the lack of real-time, eye fixation monitoring
391 during the test. While such monitoring would be desirable, it is unlikely to significantly affect the
392 peripheral thresholds reported here, particularly when the peripheral locations are selected
393 randomly during the test. The use of extended guides and appropriate fixation stimulus at the centre
394 of the screen combined with constant reinforcement to maintain fixation during the testing process
395 minimized the tendency of subjects to saccade to the peripheral stimuli. In addition, goal-directed
396 saccades towards the peripheral stimuli are best elicited with high contrast targets and are less
397 likely to occur when the stimuli are close to threshold. One of the caveats of the rod/cone flicker
398 test is that it produces large differences in sensitivity between the two photoreceptor classes (rod
399 and cone) but does not provide full isolation of rods and cones which could add to the test
400 variability. This study is preliminary and employs a small sample size. The test would need to be
401 evaluated on a larger cohort to gain greater understanding of its suitability as a functional
402 biomarker in clinical trials. Additionally, genetic testing of the participants to identify the

403 genotypes would strengthen the validation of the rod/cone test. Despite these limitations, the
404 results demonstrate that in principle, rod-enhanced and cone-enhanced stimuli can be used to
405 separate rod- and cone-mediated responses and to reveal the corresponding lack of sensitivity in
406 diseases of the retina which affect preferentially either rods or cones.

407

408 **5. Conclusions**

409 The Flicker-*plus* test can be used to quantify rod and cone-specific preferential loss of
410 sensitivity at several locations in the visual field, in patients with suspected loss of photoreceptor
411 function, without the need for dark adaptation. Notwithstanding the disease type (cone or rod-
412 dominated), both cone and rod thresholds are higher than the age-matched FMT. However, the
413 higher magnitude of photoreceptor-specific losses corresponds to the photoreceptor that is
414 predominantly affected in any particular disease. Further studies are needed to optimize the test
415 parameters for clinical use and also to investigate the usefulness of the new test in detecting
416 changes in photoreceptor sensitivities in other retinal diseases.

417

418

419 **References**

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