Structure And Function Of The Central Retina In Red Green Color Vision Deficiency: An OCT And Mf-ERG Study

Kırmızı-Yeşil Renk Körlüğünde Santral Retinanın Yapı ve Fonksiyonu: OKT ve Mf-ERG Çalışması

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ABSTRACT

Aim: This study was designed to investigate whether the structure and electrical potential of the central retina in subjects with red-green color vision deficiency (RG-CVD) differ from normals, or not.

Methods: Thirty-four male adults with RG-CVD and 34 age-matched male trichromat controls were enrolled. They had full vision and no systemic and ocular diasase except refractive errors within ±3 diopters. In addition to complete ophthalmic exam, they underwent central foveal thickness (CFT) and retinal nerve fiber layer (RNFL, overall global and 4 quadrants) thickness measurements by optical coherence tomography (OCT, Spectralis®, Heidelberg), and multifocal electroretinography (mf-ERG, Retiscan®, Roland). Results in the groups were compared. Five chronic smokers were excluded from the RG-CVD group.

Results: The mean age was 21.51±2.30 years in RG-GVD group and 22.17±2 in controls (p=0.248). CFT and RNFL measurements were similar in both eyes of subjects in both groups (p>0.05 for all). Remarkably, though not all were statistically significant, amplitudes of both N1 and P1 were higher and latencies of both N1 and P1 were shorter in all hexagons in RG-CVD cases, except for the lower P1 and N1 amplitudes found in the most peripheral one.

Conclusion: Subjects with RG-CVD seem to have normal microstructure, but higher electrical potentials with faster activity in the central retina.

Key words: *color vision deficiency; macula; multifocal electroretinography; optical coherence tomography*

ÖZ

Amaç: Kırmızı-yeşil renk körlüğü (KY-RK) olan hastaların santral retina yapı ve elektriksel potansiyelinin normal bireylerden farklı olup olmadığını araştırmak.

Method: Otuzdört yetişkin erkek KY-RK hastası ve benzer yaştaki 34 trikromat yetişkin erkek çalışmaya alındı. Tüm bireylerin ±3 dioptri refraksiyon kusuru dışında görme keskinliği tamdı ve sistemik veya okuler başka hastalıkları yoktu. Tüm bireylere tam oftalmik muayeneye ilave olarak santral fovea kalınlığı (SFK), retina sinir lifi tabakası (RSLT, global ve 4 kadranda) kalınlığı ölçümleri optik kohorens tomografi (OCT, Spectralis®, Heidelberg) ve multifokal elektroretinogram (mf-ERG, Retiscan®, Roland) ölçümleri alındı. Sonuçlar gruplarla karşılaştırıldı. KY-RK grubundan 5 kişi kronik sigara içicisi olduğundan çalışmaya alınmadı.

Bulgular: Ortalama yaş sırasıyla KY-RK grubunda 21.51±2.30 kontrol grubunda 22.17±2 bulundu (p=0.248). SFK ve RSLT kalınlığı ölçümleri arasında iki grup arasında fark saptanmadı (p>0.05). İstatistiksel olarak önemli olmasa da en periferdeki N1 ve P1 amplitüdleri dışında, KY-RK grubunda tüm hekzagonlarda N1 ve P1 amplitüdleri daha yüksek latansları daha kısa saptandı.

Sonuç: KY-RK hastalarında santral retina mikroyapısı normal olmakla birlikte yüksek elektriksel potansiyel ve daha hızlı aktivite saptandı.

Anahtar kelimeler: renk körlüğü; makula; multifokal elektroretinografi; optik kohorens tomografi

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1. INTRODUCTION

Color vision is an essential part of the human vision, and color vision deficiency is a prevalant disease. The most prevalant, red-green color vision deficiency (RG-CVD) is genetically determined by X-linked recessive inheritance and hence occurs in especially caucasian males.[1] This mild form of color vision deficiency may not disturb daily life, however, may have hazardous implications in professional life.[2]

In recent years a great interest has been raised to understand functional and structural changes in the retina in color vision deficiencies. Barthelmes et al[3] studied the structural changes in the central retina with optical coherence tomography (OCT) in patients with blue cone monochromatism and achromatopsia, and reported a highly altered foveolar structure in both diseases. Varsanyi et al[4] reported reduced thickness of the central retina on OCT in patients with achromatopsia. Gupta et al[5] reported a combined evaluation of central retina with OCT and microperimetry in patients with RG-CVD. They made a complex morphometric analysis by measuring different layers of central retina, however, they did not find significant structural differences in RG-CVD subjects when compared to healthy controls except for a narrower foveal pit on OCT. Similarly microperimetry revealed no difference between RG-CVD subjects and controls. Yılmazbas et al[6] measured the thickness of retinal nerve fiber layer (RNFL) on scanning laser polarimetry, and found no difference between RG-CVD subjects and healthy controls. This study aims to investigate structural and functional changes in the macula of subjects with RG-CVD by OCT and mf-ERG.

2. MATERIAL and METHODS

Thirty-four male subjects with RG-CVD and 34 age/sex matched healthy trichromat controls who had undergone complete ophthalmic examination including OCT and mf-ERG at our department between July 2012-September 2012, were enrolled. Five from RG-CVD group were excluded due to chronic cigarette smoking, yielding a total of 29 patients with RG-CVD. All participants were applicants for a position in Turkish Armed Forces and already had undergone full systemic evaluation before eye examination. Hence, all had full vision without any other systemic disorder or ocular disorder except for the presence of ≤ 3 dioptri spheric and ≤ 1 dioptri cylindric refraction.

The research followed the tenets of the Declaration of Helsinki. The protocol was approved by the local institutional review and ethical boards. Upon recruitment, informed consent was obtained from each subject enrolled in the study.

2.1.Detection of color vision deficiency

The color vision deficiency was initially determined using the 24-plate Ishihara's Test of Color Vision as previously described. [7] If only 9 or less plates are read correctly, the color vision was regarded as RG-CVD and underwent further confirmation with FM 100 hue test. None of the patients had experienced the FM 100 hue test before. Monocular testing was carried out and no near correction was needed as all were under 30 years of age. The caps were presented to the subjects in four sets of 21 or 22 caps as described by Farnsworth. In all subjects, an automatic electronic system calculated the total error score. The total error score was judged as abnormal if it fell outside the 95th percentile for age, as.[8]

2.2.Mf-ERG recording

The Roland-Consult RetiSCAN System (Wiesbaden, Germany) was used for the mf-ERG recording by a previously published method from our center in accordance with International Society for Clinical Electrophysiology of Vision recommendations. [9-10]

For the purpose of data analysis, the first order Kernel response was examined. We analyzed the averaged response obtained from five concentric annular retinal regions (rings) centered on the fovea: that is, the central hexagon (CH; central 6°) and four concentric rings (ring 1 [R1; 7°–12°], ring 2 [R2; 13°–18°], ring 3 [R3; 19°–24°], ring 4 [R4; 25°–30°]). We defined the first negative and positive deflections of the mf-ERG as N1 and P1, respectively. The amplitude of N1 [N1(μV)] was measured from the baseline to the first negative peak. The amplitude of P1 [P1(μV)] was measured from the first negative peak to the first positive peak. The latencies of N1 [N1(ms)] and P1 [P1(ms)] were defined as the time period from the stimulus to the peak of N1 and P1 responses, respectively.

2.3.Spectralis OCT measurement of CFT and RNFL thickness

All RNFL thickness measurements were made with the Spectralis OCT using a circular scan pattern (Spectralis software version 4.0) by an experienced technician with the attendance of one of the researchers. The scan circle was 12 degrees in diameter and the Spectralis OCT calculated the average RNFL thickness for the overall global, and for 4 quadrants (nasal [$RNFL_N$], superior [$RNFL_S$], temporal [$RNFL_T$], and inferior [$RNFL_I$]). The scans with good signal strength (15 or higher) were included.

All CFT measurements were performed by measuring the distance between the vitreoretinal interface and the inner edge of the retinal pigment epithelium. All these measurements were taken separately in right as well as left eyes of RG-CVD subjects and controls.

2.4.Statistical analysis

Statistical analyses were performed using SPSS (Statisti-

cal Package for Social Sciences, version 15.0, Chicago, IL, USA). As the variables were expressed as median (minimum-maximum). A *p* value less than 0.05 was accepted significant. The data were evaluated and found not to be normally distributed. Therefore a Mann–Whitney U-test was used for comparison.

3. RESULTS

The mean age of the cases was 21.51±2.30 years and the mean age of controls was 22.17±2.22 years. There was no statistically significant difference between the two groups regarding age (p=0.248).

Table 1 and 2 show the median CFT and the median thickness of RNFL for both eyes in RG-CVD cases and controls on OCT. When compared between cases and controls, the difference in the CFT and thickness of RNFL overall and RNFL in 4 quadrants (nasal, superior, temporal and inferior) was not statistically significant (p>0.05 for all).

Figure 1 shows the summed mf-ERG responses (CH and R1-4) in the right eye of RG-CVD subjects and controls. When compared between cases and controls, the difference in P1 (p<0.001) and N1 amplitudes (P=0.011) in CH; P1 amplitude (p<0.001), P1 (p=0.016) and N1 latencies (p<0.001) in R2; P1 amplitude (p=0.001) and P1 latency (p<0.001) in R3; P1 amplitude (p=0.01) and P1 latency (p=0.003) in R4; P1 latency (p=0.02) in R5 was statistically significant. However, generally amplitudes were higher and latencies were shorter in RG-CVD cases than controls in all rings except for the lower P1 and N1 amplitudes found in R5.

Figure 2 shows the summed mf-ERG responses (CH and R1-4) in the left eye of RG-CVD subjects and controls. When compared between cases and controls, the difference in P1 amplitude (p=0.02), N1 amplitude (P=0.034) and P1 latency (p=0.004) in CH; P1 amplitude (p<0.001), P1 (p<0.001) and N1 latencies (p<0.001) in R2; P1 amplitude (p=0.004), P1 (p<0.001) and N1 latencies (p=0.009) in R3; P1 amplitude (p=0.015), P1 (p<0.001) and N1 latencies (p=0.031) in R4; P1 latency (p=0.002) in R5 was statistically significant. However, similar to right eye amplitudes were higher and latencies were shorter in RG-CVD cases than controls in all rings except for the lower P1 and N1 amplitudes found in R5.

4. DISCUSSION

Interestingly we recorded symetrically higher mf-ERG responses in RG-CVD cases than controls except for R5. Though we don't have an exact explanation, It is evident that these individuals have different electrical activity from healthy subjects in the central retina due mostly to the cone dysfunction. The OCT was widely used to provide unanticipated pathological insights into the cone disorders. Michaelides et al [11] investigated the integrity of cone photoreceptor mosaic with adaptive optics and its structural correlates with Spectralis OCT in four cases with oligocone trichomacy, characterized by normal or near-normal color vision despite an absent or reduced cone electroretinogram. They found that both cone density and cone distribution were significantly disrupted in three cases while they were preserved in a male patient. Supportingly, a marked reduction in central retinal thickness was observed in the three patients contrary to the male patient. Thus they suggested a new way of classifying for this entity into oligocone trichomacy and oligocone trichomacy-like phenotypes. In contrast, Andersen et al. obtained OCT images in four individuals and found mildly reduced retinal thickness in the parafoveal and perifoveal regions while normal in the center.[12] Barthelmes et al[3] in a study

Table 1. Right eye comparison for CFT and RNFL thickness on OCT (median; min-max)

	CFT	$RNFL_{average}$	$RNFL_{nasal}$	$RNFL_{superior}$	$RNFL_{temporal}$	$RNFL_{inferior}$
RG-CVD	213(170-251)	114.18(93.07-130.33)	90(56-116)	140(110-174)	78(58-107)	139(105-183)
Control	211(176-284)	114.76 (100.67-137.64)	84(52-130)	137(79-178)	77(49-119)	139.5(97-180)
p	0.291	0.248	0.662	0.684	0.544	0.994

CFT, Central foveal thickness; RG-CVD, Red green color vision deficiency; RNFL, Retinal nerve fiber layer

Table 2. Left eye comparison for CFT and RNFL thickness on OCT (median; min-max)

	Central Foveal Thickness	$RNFL_{average}$	$RNFL_{nasal}$	$RNFL_{superior}$	$RNFL_{temporal}$	$RNFL_{inferior}$
RG-CVD	219(172-263)	110.12(94.66-136.71)	85(52-126)	137(113-175)	81(57-130)	134(102-183)
Control	218(177-298)	115.8(100.67-134.09)	82.5(53-129)	142.5(72-181)	80.5(60-110)	142.5(96-173)
p	0.503	0.208	0.684	0.988	0.927	0.636

CFT, Central foveal thickness; RG-CVD, Red green color vision deficiency; RNFL, Retinal nerve fiber layer

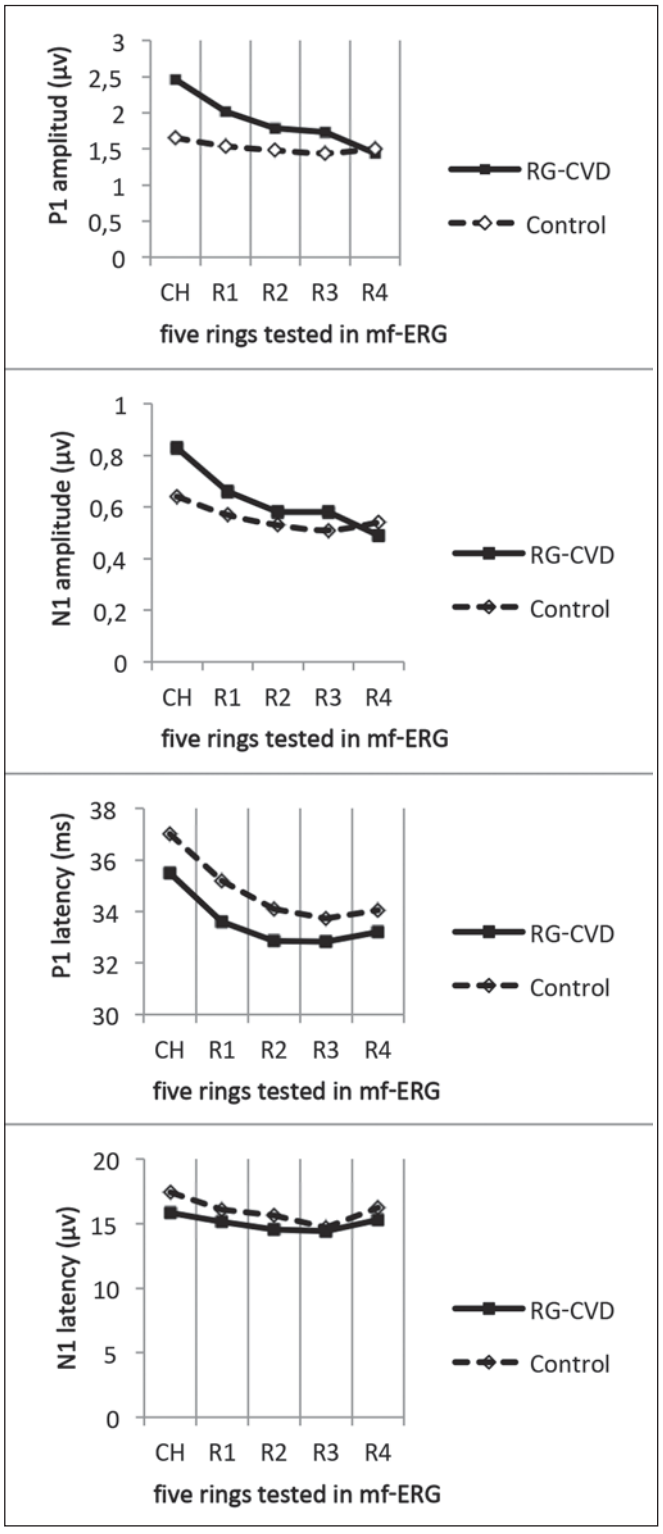

Figure 1. *Right eye comparison for the summed mf-ERG responses obtained from five rings between RG-CVD subjects and control*

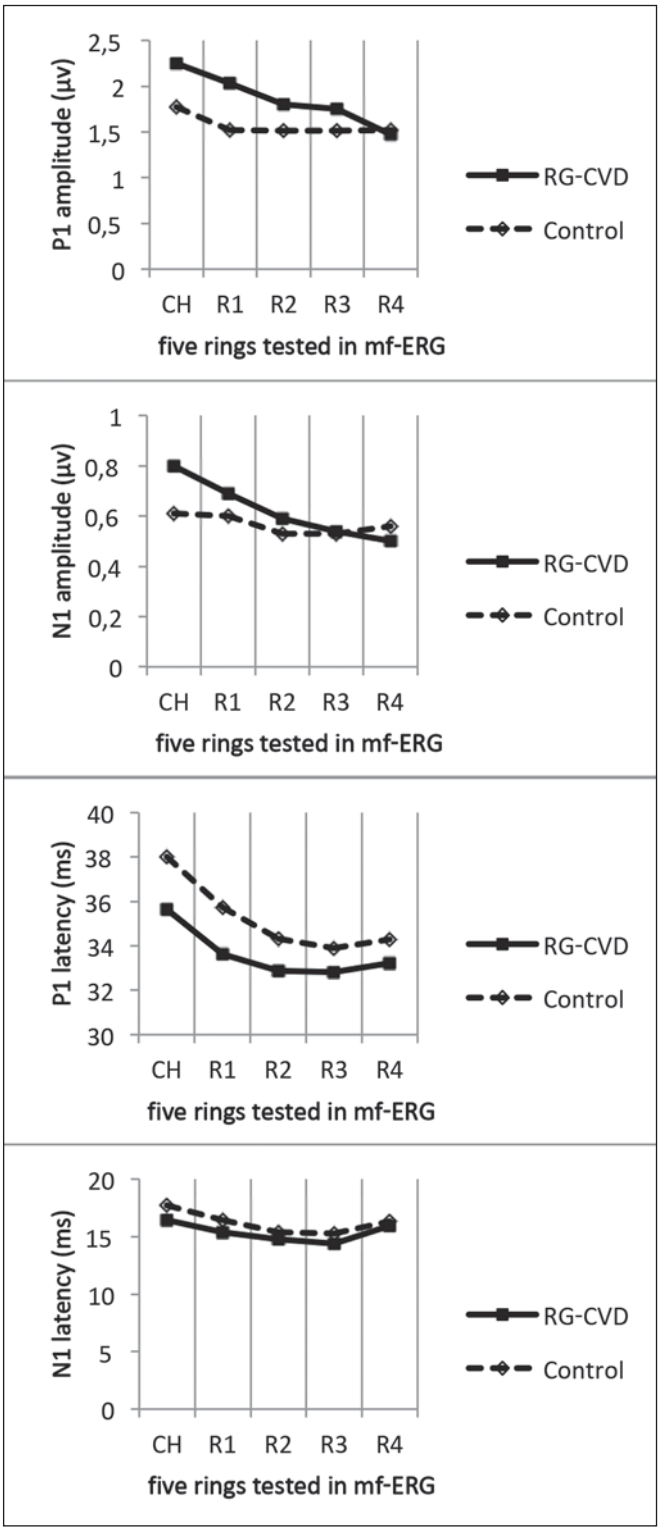

Figure 2. *Left eye comparison for the summed mf-ERG responses obtained from five rings between RG-CVD subjects and controls*

including patients with blue cone monochromatism and Varsanyi et al [4] in a study including patients with achromatism found significantly reduced CFT on OCT.

There are few studies investigated the structural changes in the retina of subjects with RG-CVD. Carroll et al[13] found reduced or normal outer nuclear layer. Gupta et al identified and measured the thickness of the various retinal layers (total retinal thickness, thickness of outer nuclear layer, thickness of photoreceptor layer, vertical thickness of outer segment and inner segment of the photoreceptors, horizontal diameter of the outer segment of the photoreceptors) at the central fovea and at additional 10 points, five on each side of the foveal center, in cases with RG-CVD and con-

trols. [5] However, the comparison between groups was unrevealing except for the relatively narrower outer segment horizontal diameter and foveal pit. They related this finding with the absence of central ward migration of cones which is believed to commence before birth and complete in the early months of life. [14-15] Hence, they hypothesed that subjects with RG-CVD may have smaller number or smaller diameter of red green cones in the fovea. However, this theory had been discontinued since the introduction of the study by Carroll et al.[16] They investigated two RG-CVD cases with molecular genetics, adaptive optics retinal imaging and retinal densitometry, and compared results with a trichomat. These two dichromats had distinct genotypes with distinct phenotypes. In the first case the L gene was by replaced by another encoding an M photopigment, which resulted in replacement of L cones by M cones with normal number of functional cones in the central retina finally. However, in contrast, in the second case M gene was replaced by a nonfunctional one with a resultant patchy loss of normal cones throughout the photoreceptor mosaic. Moreover, In two dichromats and one trichromat cone density in the center of the fovea was similar, which suggested that normal central ward migration of the cones occurs in dichromats and they begin to degenerate later. In the dichromat with M cone degeneration no deficit other than a loss of color vision in clinical tests was present, which supported a highly resistant visual system able to tolerate loss of sampling elements to some extent.

To date no clear correlation was established between structural and functional findings in RG-CVD. The largest study by Gupta et al found narrower outer segment diameter on OCT and tried to relate this with smaller or lower number of foveal cones, however, no functional correlate was reported. [5] Though we did not evaluate in a similar detailed manner on OCT, we observed no difference in CFT and thickness of RNFL measurements between RG-CVD cases and controls. The recording of higher mf-ERG responses in central five rings is not correlated with the idea that normal central ward migration of foveal cones is absent in these patients. Conversely, when combined with the responses obtained from CH and R1-4, the recording of lower responses in R5 may indicate a farther central migration of cone plates in these patients. However, long time ago, some studies based on frequency of seeing curves reported that the packing of foveal cones in dichromats is comparable to that in trichomats. [17-18] It is not wise to make a head to head comparison between these studies as functional sensitivity differs much between these techniques. A second hypothesis may be that high plasticity of human visual system may cause overfunctioning in one way while underfunctioning in the other. In this context, our findings which suggest neural plasticity in the retina namely in the first neuron of visual pathway may pave the way for a new way of thinking.

Chromatic signals follow different pathways from retina to the cortex. The signals of the three types of cones (small [S], medium [M] and long [L] cones according to their peak sensitivity) are combined in the retina in two chromatic channels, S-(L + M) and L-M, that are transmitted in two distinct pathways to the cortex: S-(L + M) is signaled by the koniocellular pathway and L-M is signaled by the parvocellular pathway. [19] As can be seen in the structural studies aforementioned above, there is no clear structural difference between RG-CVD cases and controls. Therefore, structural studies limited to retinal level may not explain the entire pathology embeded in RG-CVD. Baseler et al [20] showed that pronounced cortical changes may ocur in patients with congenital photoreceptor abnormality such as rod monochromatism. Thus further studies covering the whole visual circuitry may help much in explaining the fundamental pathology in RG-CVD.

Gupta et al [5] used microperimetry to assess macular function in RG-CVD cases. The functions of auto-tracking and automatic registration of one test to another allow for precise determination of central retinal sensitivities. However, it has a narrower range of stimulus intensities and a dimmer background luminance which results in a lower dynamic range (14-34 dB) when compared to automated perimeter. Moreover, with the addition of "ceiling effect" occuring at the top of its retinal sensitivity threshold subjects with healthy macula often record thresholds of 20 dB across the entire macula, without discerning small differences in sensitivity between the fovea and parafovea. [21] On the other side, mf-ERG allows capturing the bioelectric responses from different retinal areas in particular from the macular region, which are mainly driven by the preganglionic components. [22] Furthermore, the first-order kernel of mf-ERG primarily stems from photoreceptors and bipolar cells . [23] Ring analysis allows separating selective measures of bioelectrical responses obtained from different locations on macula. [24-25] With this regard mf-ERG seems more sensitive in discerning small deviations from normal. However, when a ring analysis is performed, individual hexagons are not actually evaluated singly. [23,26] Therefore, some individual abnormal responses could be masked by the adjacent normal responses with resultant normal averaged responses obtained in the entire tested area. In this case control study matched for age and sex, we excluded chronic smokers since nicotine has been shown to affect macular functions. [10] There are some shortcomings of our study; first we had a small sample size and no genetic testing was performed to see variations among RG-CVD cases; second, we did not perform a intraretinal layer analysis on Spectralis OCT, which would give direct correlation between mf-ERG and its main structural source photoreceptor layer. On the other hand all the subjects were fully motivated during the tests as they were applicants for a position in Turkish Armed Forces.

In conclusion, we observed normal thickness of central fovea and RNFL, however, surprisingly high bioelectrical activity of central retina in RG-CVD cases. Though we don't have an exact explanation, two hypotheses can be postulated; farther central migration of cone photoreceptors or overfunctioning of cones in bioelectrical activity while underfunctioning in color perception, which suggest retinal functional remodeling by neural plasticity in RG-CVD. However, further studies are needed to prove these observations.

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