Retrospective evaluation of pre-surgical electroretinography results in a mixed-breed canine population presented for cataract removal surgery

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Michaela L Wegg[1], Danica Pollard[2] and Ron Ofri[3]

1. The Roslin Institute, The University of Edinburgh, Easter Bush Campus, Midlothian, EH25 9RG, weggmichaela@gmail.com
2. The Rodhams, Rodham Road, Wisbech, PE14 9NU
3. Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Rehovot 7610001, Israel

ABSTRACT

OBJECTIVE

Electroretinography (ERG) is used prior to cataract removal surgery to assess retinal function. We aimed to replicate and improve upon previous studies by performing a full ECVO protocol and by examining the retina post-surgery in all patients.

ANIMALS STUDIED

127 eyes from 67 dogs were included in the study.

PROCEDURES

A full ECVO protocol electroretinography, which includes extensive rod and cone analysis, was performed on all dogs presenting for cataract surgery.

RESULTS

Our main findings were that amplitudes, but not implicit times of rod responses decreased with advanced cataracts. Amplitudes of the single flash rod and rod flicker responses were significantly lower in eyes with mature cataracts, and the former also decreased in hypermature cataracts. Cone flicker amplitude responses were also significantly lower in eyes with mature and hypermature cataracts. However, mixed single flash rod-cone and cone responses, with the exception of the mixed rod-cone a-wave amplitude in eyes with hypermature cataracts, were unaffected by cataract stage. The b-wave amplitude of the scotopic, mixed rod-cone, and photopic cone responses were affected by age and decreased by an average of 2.9, 7.5, and 1.5μV/year, retrospectively ($p < 0.01$).

CONCLUSIONS

Lower ERG amplitudes in canine cataract patients may result from aging or the presence of advanced cataracts and may not indicate the presence of retinal disease.

Keywords: ERG, electroretinogram, canine, dog, cataract, ageing

INTRODUCTION

Electroretinography (ERG) is a non-invasive diagnostic modality used to record retinal responses to light stimulus. The most commonly used stimulus is flash, allowing the recording of full-field ERG (fERG). By changing various parameters of the stimulus, such as its intensity, frequency, and pre-recording ambient light levels, one can adapt the protocol to preferentially record rod, cone, or inner retinal function, and diagnose many retinal diseases. [1-3]

In 2002, the first guidelines for clinical ERG recording in the dog were published.[4] These guidelines, and their 2012 update,[5] recommend the use of two protocols in canine patients. The first, long (32 min or more) protocol is an exhaustive test of rod and cone function, which is recommended for the early diagnosis of inherited retinal degenerations and dystrophies, most commonly various forms of rod-cone degeneration.[6-8] However, it is also used in the diagnosis of less common diseases such as achromatopsia[9,10] and congenital stationary night blindness.[11,12] The second, brief (5 min) protocol, may be used in the diagnosis of acute cases of blindness, such as differentiating between optic neuritis and Sudden Acquired Retinal Degeneration Syndrome,[13,14] and to determine the prognosis of retinal reattachment surgery.[15] However, its most common indication is the pre-operative determination of retinal function prior to cataract surgery. As the presence of a surgical cataract prevents a comprehensive ophthalmoscopic examination of the retina, a pre-operative ERG recording is indicated to rule out the presence of blinding retinal disease in the patient and to determine whether surgery will be beneficial.[16,17] Indeed, it has been demonstrated that in a brief protocol recording b-wave amplitudes of 78.5µV (or lower) had high sensitivity of 100% (95% CI: 87.2–100%) and high specificity of 96.7% (95% CI: 88.4–100%) in determining retinal disease and retinal detachment in dogs with

cataracts.[18] Therefore, it is not surprising, that in a study of 204 cataractous eyes, scheduled for phacoemulsification surgery, that 51 of these eyes (which could not be examined ophthalmoscopically due to the presence of cataract) Progressive Retinal Atrophy was demonstrated by ERG.[19]

However, it is possible that an ERG recording in a cataractous eye will be affected by the presence of the cataract itself, rather than by retinal disease. For example, cataracts cause light scattering, which may have a significant effect on the flash stimulus used to elicit the ERG.[20] Alternatively, the presence of advanced cataracts may cause secondary lens-induced uveitis (LIU), which could likewise affect the ERG.[21] However, as previous authors do not report on the results of the post-operative ophthalmoscopic examination, nor on the post-operative vision of the patients, it is difficult to determine whether some of their findings may be attributed to the presence of retinal disease. Therefore, we aimed to replicate and improve upon their study by recording pre-operative ERG in cataractous dogs using a more comprehensive protocol (i.e., the published "long" protocol) [4,5] and performing a retinal examination of the patients after surgery.

MATERIALS AND METHODS

1. Animals

Dogs were selected for this study if they presented to Rowe Referrals (Bristol, Great Britain) for cataract assessment and an ERG recording between August 2017 and February 2019. All animals underwent a full ophthalmic examination, including slit-lamp biomicroscopy (SL-17, Kowa), rebound tonometry (TonoVet, ICare®), and when

possible indirect ophthalmoscopy (Vantage Plus, Keeler and Volk) by either a European College of Veterinary Ophthalmologists (ECVO) Diplomate or a certificate holder in veterinary ophthalmology. Eyes with cataracts were assigned into one of the following groups, based on the cataract stage: [16,17]

1. Incipient - opacity involving less than 15% of the lens volume.
2. Immature - opacity involving 15 – 99% of the lens volume, the lens is not totally opaque and some tapetal reflection is visible.
3. Mature - a complete cataract where there is no tapetal reflection or menace response.
4. Hypermature - the lens volume is reduced, water clefts, dense white refractile plaques, and wrinkling of the lens capsule are visible.

Eyes (of unilaterally-affected dogs) with no evidence of cataract, in a single eye, were assigned to a control group. Bilaterally affected dogs had eyes allocated to the correct group based on the cataract stage as defined above. All eyes with immature, mature, and hypermature cataracts were scheduled for surgery and underwent a pre-operative ultrasound to rule out retinal detachment and vitreal disease. Eyes with retinal detachment, progressive retinal atrophy (PRA) (diagnosed following an ERG recording or examination of the contralateral, non-cataractous eye), corneal pathology, which prevented clear visualization of the lens, or glaucoma were excluded from the study. Likewise, all eyes with pre-operative lens capsule rupture were excluded, due to the resulting severe LIU. All other cases of LIU were medically treated prior to the pre-operative ERG recording and surgery. Patients that did not receive an ophthalmic and fundic examination 12 hours following surgery, daily until discharge and 1-week post-surgery were also excluded. Signed consent was collected for all the procedures.

2. Electroretinography

ERG was recorded in all study eyes at least 60 min after the ophthalmic examination, to allow for recovery of dark-adapted responses.[22] ERG responses were recorded simultaneously in both eyes of all study dogs using the RetiPort System (An-vision, Hennigsdorf, Germany). The ERG was recorded in both eyes of all patients, including, in patients, with no cataract or incipient cataract in one eye. The eye with the more advanced cataracts were scheduled for surgery. Animals were prepared for the ERG recording in a lit room with an ambient illumination of 155 lux (measured using MT940, Major Tech). Pupils were dilated with 0.5% tropicamide and 20 min later the animals were sedated with an intravenous injection of 25µg/kg of medetomidine hydrochloride (Sedator™ 1.0mg/ml, Dechra) and 0.1mg/kg butorphanol (Torbugesic™ 10mg/ml, Zoetis). Animals were positioned in sternal recumbency, eyelids retracted using Barraquer eyelid retractors, and dorsally positioned stay sutures were placed in the bulbar conjunctiva to keep the pupils centered. Kooijman contact electrodes with a built in 4W white LED stimulator served as both active electrodes and Ganzfeld stimulator. [23-25] To improve conduction, these were coupled to both corneas with a 0.2% carbomer gel (Clinitas Hydrate™, Altacor). Subcutaneous needles served as reference and ground electrodes and were placed 4cm from the lateral canthi of both eyes and at the occipital protuberance, respectively. [26] Impedance was kept under 5KΩ.

Five minutes after the onset of sedation the room lights were turned off (illumination 0 lux) and a standardized, "long" ECVO protocol was recorded from both eyes. [4,5] This included a 20-minute dark adaptation period, during which the scotopic single flash response was recorded every four minutes (total of 6 recordings, starting at time 0) using a dim stimulus (average of four traces, 0.2 Hz, 0.03 cd·s /m^2). Next, the rod flicker response (average of five traces, 8.9 Hz, 0.03 cd·s /m^2) was recorded, followed by the

mixed rod-cone responses to a standard flash (average of four traces, 0.06 Hz, 3 cd·s /m^2). Cone function was recorded following 10 minutes of light adaptation (30 cd/m^2) using a high intensity flash (average of 15 traces, 2 Hz, 3 cd·s /m^2) to generate the single flash photopic response, and the cone flicker response (eight traces, 27.8 Hz, 3 cd·s /m^2). Following the recording, the sedative drugs were reversed with atipamezole hydrochloride 0.125mg/kg (Antisedan™ 5mg/ml, Vetoquinol) given intramuscularly.

All animals had a full ophthalmic examination, including fundoscopy, 12 hours after surgery. Dogs were re-examined daily until discharged, one week post-operatively, and subsequently subject to individual status.

3. Statistical Analysis

Amplitudes for the a-wave and b-waves were measured from baseline to the first trough and from that trough to the next positive peak, respectively. Implicit times, which are the respective time intervals between the stimulus onsets to the trough or to the positive peak, were measured to examine the response kinetics. Data were stored in a Microsoft Excel spreadsheet and imported into software Stata (IC v 13.0) for coding and statistical analyses. The demographics of the sampled dogs were described.

Normality of data were assessed using a combination of histograms with overlaid kernel density plots and the Shapiro-Wilk test. Due to the majority of continuous variables being non-normally distributed, they were described as medians with interquartile range (IQR) and range. Categorical variables were summarized as proportions and percentages.

All statistical analyses were conducted at the individual eye level, and it was assumed that individual eyes were independent of each other. Overall comparisons across cataract stages and dark adaptation times for non-parametric data, including a- and b-

wave amplitudes and implicit times for all 10 responses of each eye, were analyzed using the Kruskal-Wallis test where $P<0.05$. A posthoc Dunn's test with Sidák adjustment (to adjust for multiple comparisons) was used to assesses pairwise comparisons between control and cataract groups.

Mixed-effects linear regression modelling, with dog ID as a random effect, was used to identify differences in a- and b-wave amplitudes and implicit times for all 10 responses of each eye across cataract types while adjusting for other variables such as age (tested as both a continuous variable and re-categorized into age categories based on quartiles), breed, sex and side (right or left eye). Data that were considerably skewed (the b/a ratio of the mixed rod-cone and photopic cone responses) were log transformed to create a normal distribution for linear regression modeling. Predictive coefficients estimated by the linear regression modeling are presented with 95% confidence intervals (CI). Multivariable models were built using manual, stepwise backward elimination with variables retained in the model where the likelihood ratio statistic $p < 0.05$.

RESULTS

1. Animals

In total 67 dogs were included in the study, with 125 eyes available for analysis. Nine patients had unilateral recording due to previous enucleation. There was an approximately equal sample of male (49.3%, n = 33) and female (50.7%, n = 34) dogs. Neutering status was known for 64 dogs. Of these 32.8% (n = 21) were neutered males, 39.0% (n = 25) were neutered females, 18.8% (n = 12) were intact males, and 9.4% (n = 6) were intact females. The most frequently represented breeds were Bichon Frisé (20.9%, n = 14), Labrador retrievers (7.5%, n = 5), Jack Russel terriers (4.5%, n = 3), and other terrier breeds (13.4%, n = 9). Of the study population, 8.9% (n = 6) of dogs

were classified as extra small (< 5kg), 50.8% (n = 35) as small (6-10kg), 20.9% (n = 14) as medium (11-26kg) and 19.4% (n = 12) as large (27-45kg). The median age of the dogs in the study was 6.4 years (IQR 2.7 to 9.0 years, range 0.4 to 12.4 years) across all cataract groups.

Each eye was placed into a group and was assessed within its cataract group. Of the 125 recorded eyes, 17 (13.6%) were diagnosed with incipient, 28 (22.4%) with immature, 44 (35.2%) with mature and 27 (21.6%) with hypermature cataracts. Nine eyes (7.2%) of dogs that presented with unilateral cataracts had no lenticular opacities, in the other eye, and these served as control eyes. The median ages of the control, incipient, immature, mature, and hypermature eyes were 2.8 years (IQR 1.3 to 6.3 years), 4.4 years (IQR 0.9 to 7.0 years), 6.6 years (IQR 3.5 to 10.1 years), 7.5 years (IQR 3.6 to 9.0 years) and 6.4 years (IQR 5.0 to 10.3 years), respectively.

Some patients had missed recordings in full ECVO protocol due to electromagnetic artefacts and had to be excluded from the study. These differences have been highlighted in the numbers in Table 1. Although control eyes generally belonged to younger dogs, no significant difference in median age across the five groups was identified (Kruskal-Wallis $p = 0.120$).

All the dogs were sighted immediately after surgery based off a positive menace test and the ability to navigate the unfamiliar hospital setting. The pupillary light reflexes were not assessed post-surgery due to the use of pharmacological agents during and after the surgery which would have altered this response and given erroneous results. They also had a full ophthalmic examination within 24 hours of the surgery and no abnormal retinal changes were seen on indirect ophthalmoscopy.

2. Electroretinography

Table 1 details the number of control and cataractous eyes in which each of the ERG responses were recorded. Figure 1 presents scotopic, mixed rod-cone, and photopic responses recorded from representative eyes in the five groups.

The median and mean implicit times and amplitudes of the dark-adapted rod responses in the five clinical groups (*i.e.,* control eyes and the four cataract groups) are presented as box and whisker plots in Figure 2 and as numerical values in the supplementary data.

2.1 Rod (scotopic) Response

Analysis of the scotopic, single flash rod responses for all clinical groups combined showed a significant difference ($p < 0.001$) between implicit times of the responses at the start of the dark adaptation period (DA1) and each subsequent 4-minute interval (DA2 to DA6) during 20 min of dark adaptation (Table 2). There was also a significant difference in timepoints DA1 and DA2 in the scotopic b-wave amplitude, when all groups were compared (Table 2). As expected, amplitudes of the single flash rod responses increased with time in the dark across all five clinical groups although differences between the amplitudes of the different groups did not reach significance when the later dark adaptation periods were compared with each other (between 8 minutes [DA3] and 20 minutes [DA6]) (Table 2).

A comparison of the scotopic, single flash rod responses between the five clinical groups shows that implicit times were mostly unaffected by the presence of cataract and cataract stage, with only three significant differences in all of the multiple comparisons: those being between the control and incipient group at DA2 ($p = 0.02$), the incipient and mature groups at DA5 ($p=0.003$) and the incipient and mature groups at DA6 ($p=0.03$) (Figure 2A). On the other hand, amplitudes of the single flash rod

responses were affected by cataract stage in all but the first time period (DA1), with significantly lower amplitudes in most recordings of eyes with mature and hypermature cataracts when compared to the other groups (Figure 2B).

Implicit times of the rod flicker responses were not affected by the cataract stage ($p > 0.25$). The amplitudes of the rod flicker responses tended to decrease in advanced cataracts, and there was a significant decrease in amplitude between the incipient and mature cataract groups (Figure 3A; $p = 0.014$). The scotopic b-wave amplitude responses were further affected by the clinical group, with each cataract stage having a significantly lower amplitude in comparison to the control group ($p < 0.001$), and dark adaptation time (coefficient = 3.0, 95% CI 2.7, 3.4; $p < 0.001$).

2.2 Mixed rod-cone Response

Mixed rod-cone a-wave implicit times were significantly longer for the mature cataract stage (Figure 3B; $p = 0.047$) and were not affected by cataract stage in the other groups ($p = 0.18$ to $p = 0.74$). The a-wave amplitudes tended to decrease as the cataract stage advanced and were significantly reduced in eyes with hypermature cataracts compared to the control group (Figure 3C; $p = 0.04$). The mixed rod-cone b-wave implicit times and amplitudes did not differ significantly between the five clinical groups ($p > 0.2$). There were no significant differences between the five clinical groups in the b/a ratio of the mixed rod-cone responses ($p > 0.2$)

2.3 Cone (photopic) Response

Implicit times and amplitudes of the a-wave ($p > 0.05$ and $p > 0.18$, respectively) and b-wave ($p > 0.16$ and $p > 0.3$, respectively) of the light-adapted single flash photopic responses did not differ significantly between the five groups. There were no significant

differences observed in the b/a ratio of the single flash photopic responses ($p > 0.18$). Finally, cone flicker implicit times did not differ significantly between groups ($p > 0.5$). However, flicker amplitudes were significantly lower in eyes with mature ($p = 0.03$) and hypermature ($p = 0.01$) cataracts when compared to the control group (Figure 3D).

2.4 Effect of age

Implicit times of the six scotopic, dark-adapted responses were not affected by age ($p > 0.09$). However, b-wave amplitudes of the dark adaptation scotopic, mixed rod-cone and photopic cone responses were affected by age, decreasing by an average of 2.9 µV/year (95% CI -4.7, -1.0; $p = 0.002$), 7.5 µV/year (95% CI -10.8, -4.1; $p < 0.001$) and 1.5 µV/year (95% CI -2.4, -0.7; $p < 0.001$), respectively (Figure 4)**.** Consequently, the b/a ratio of the mixed rod-cone ($p = 0.001$) and photopic cone ($p = 0.03$) responses were significantly lower in the older aged dogs. Rod flicker response amplitudes were likewise significantly reduced with age (coefficient = - 1.9, 95% CI -3.3, -0.4; $p = 0.01$). For all of the results in numerical format please see the supplementary material.

DISCUSSION

Our main findings are that amplitudes, but not implicit times, of rod responses decreased in advanced cataracts. Amplitudes of the single flash rod responses were significantly lower in eyes with mature and hypermature cataracts, and those of the rod flicker responses were significantly lower in mature cataracts. However, mixed single flash rod-cone and cone responses, with the exception of the mixed rod-cone a-wave amplitude, were unaffected by cataract stage, as were the b/a wave ratios. Finally, cone flicker amplitudes were significantly lower in eyes with mature and hypermature cataracts.

Our results differ from those of Park et al (2009),[19] who found no effect of cataract stage on ERG and resemble those of Maehara et al (2007),[27] who also report that eyes with mature cataracts had significantly lower single flash rod responses, as well as mixed rod-cone a-wave and cone flicker amplitudes. Maehara et al (2007) propose that the decreased responses in eyes with mature cataracts may be because the mature cataract acts as a filter that reduces stimulus intensity. Indeed, it has been reported that the presence of cataracts may decrease flash intensity by 0.5 log units.[28] In our study all post-operative ophthalmoscopic examinations were unremarkable and all dogs were visual after surgery, suggesting that in both our study the low amplitudes recorded were due to the presence of cataract rather than retinal disease. This hypothesis could have been tested by performing post-operative ERG recordings. While such recordings have not been performed in our study, Maehara et al report significantly higher amplitudes in their post-operative recordings.[27] Similar increases in post-operative ERG amplitudes has recently been demonstrated in humans using both a comprehensive ISCEV protocol recording [29] as well as a brief flicker recording, [30] supporting the hypothesis regarding the effect of mature cataracts on ERG amplitude. Indeed, a study of human patients with unilateral mature cataracts showed significantly decreased ERG amplitudes in the affected eyes [31] compared to the unaffected eyes. Therefore, it is possible that for their pre-operative ERG recordings veterinary ophthalmologists should establish baseline values that incorporate the aggravating effect of the cataract itself, as each set-up and machine are likely to have differing values

If indeed advanced cataracts reduce the flash intensity and ERG amplitudes, one would expect such a reduction to occur in eyes with both mature and hypermature cataracts. While we observed such a decrease in both cataract categories, Maehara et al report it only in mature cataracts.[27] There are additional differences between our findings and

those of Maehara et al. They report significantly lower mixed rod-cone b-wave and single flash cone response amplitudes that we did not observe. On the other hand, we observed decreased single flash rod and cone flicker responses in hypermature cataracts, a decrease that they did not observe. One possible reason for the difference between some of the findings in the two studies may be statistical methods. As most of our data was not normally distributed, results were analyzed using non-parametric tests (Kruskal-Wallis, and subsequently the Dunn's test). Maehara et al (2007) do not report on the distribution of their data, but used a pairwise Student's t-test for normally distributed data.

Another interesting finding in our study is the age-related decline in amplitudes of many of the responses we recorded. While previous studies have reported amplitude differences between dogs from different age groups, [32] to the best of our knowledge this is the first study to show a linear decrease spanning 12 years (Figure 4). This finding is somewhat surprising, as two studies using optical coherence tomography failed to show age-related retinal thinning in adult and elderly dogs. [33,34] However, studies looking at specific retinal neuron populations have shown such a decline in humans. It has been shown that in the human retina, the concentration of cones and rods decreases at a rate of 0.18%/year and 0.37%/year, respectively.[35] Overall, the number of rods decreases by 30% by the age of 90, while the number of bipolar cells decreases by 21% and 27% at ages 35-62 and 60-90, respectively. [36,37] The inner retina is also affected, with losses of 25% of all retinal ganglion cells reported by 75 years of age. [38] Our results suggest that similar losses may occur in dogs, albeit without concurrent retinal thinning, and support previous recommendations that results of canine ERG recordings be compared to those of age-matched dogs. [4,5,32]

A limitation of this study is that some of the recordings had electromagnetic artefacts and had to be excluded from the analysis, therefore not every animal has a full set of results. If this study were to be repeated it would be performed using a faraday cage and a post operative full ECVO protocol ERG recording would be performed. The dark adaptation recordings used the 2002 recommendation of 0.03 cd·s /m^2, [4] whereas the current 2012 updated recommendations indicate that a lower value of 0.01-0.02 cd·s /m^2 should be used. [5]

In conclusion, lower ERG amplitudes in canine cataract patients may result from aging or the presence of advanced cataracts and may not necessarily indicate the presence of a retinal disease.

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The authors declare that they have no conflict of interest.

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FIGURE LEGENDS

Figure 1

Scotopic, single flash rod responses (recorded after 20 min of dark adaptation) (A), rod flicker (B), mixed rod-cone responses (C), single flash photopic response (D), and cone flicker (E) traces recorded from representative dogs with incipient, immature, mature, and hypermature cataracts, and an unaffected control eye. Horizontal bar = 50 msec in all panels.

Figure 2

Box and whisker plots of the scotopic ERG responses recorded from unaffected control eyes (dark blue), and eyes with incipient (orange), immature (gray), mature (yellow), and hypermature (light blue) cataracts. Six scotopic responses were recorded at 4 min intervals during 20 minutes of dark adaptation (DA1-DA6), and the b-wave implicit times and amplitudes are presented in panels A&B.

Figure 3

Box and whisker plots of the scotopic ERG responses recorded from unaffected control eyes (dark blue), and eyes with incipient (orange), immature (gray), mature (yellow), and hypermature (light blue) cataracts.

Figure showing the recording of the rod flicker amplitude response is presented in panel A. Mixed rod-cone responses to 3 cd·s /m^2 flashes recorded after 20 min of dark adaptation. Implicit times and amplitudes of the a-wave are presented in panels B&C. The photopic flicker b-wave amplitude response to 3 cd·s /m^2 flashes (27.8Hz) recorded after 10 min of light adaptation is presented in panel D.

In the box plots, the boundary of the box closest to zero indicates the 25^{th} percentile, a black line within the box marks the median, the X marks the mean, and the boundary of the box farthest from zero indicates the 75^{th} percentile. Whiskers above and below

the box indicate the 10th and 90th percentiles. Points above and below the whiskers indicate outliers outside the 10th and 90th percentiles. Significant differences in values are indicated by a horizontal bar and a p-value < 0.05.

Figure 4

Linear regression graphs showing the effect of age on the b-wave amplitudes of the scotopic (A), mixed rod-cone (B), and photopic cone responses (C). A line of best fit and a shaded area highlighting the 95% confidence interval is also shown.

Table 1. Recorded eyes

Table presents the number of control/cataractous eyes in which each of the ERG responses was recorded (DA-dark adapted)

Recording	Control	Incipient	Immature	Mature	Hypermature	Total
DA1	9	16	28	43	25	121
DA2	9	17	28	43	25	122
DA3	9	16	29	43	25	122
DA4	9	17	28	43	23	120
DA5	9	17	24	40	23	113
DA6	9	16	23	38	23	109
Rod flicker	7	16	27	40	25	115
Mixed rod cone response	9	17	28	44	25	123
Photopic	9	15	25	40	24	113
Cone flicker	9	16	26	41	27	119

DA Dark adaptation, where DA1 is 0 minutes, DA2 is 4 minutes, DA3 is 8 minutes, DA4 is 12 minutes, DA5 is 16 minutes and DA6 is 20 minutes

Table 2. Posthoc comparison using Dunn's test with Sidák adjustment of median B-wave implicit times (ms) and amplitudes (μV) for all clinical groups combined across the dark adaptation period.

*shows median difference is significant at the 0.05 level between this time period and all other time periods. DA- Dark adaptation

Dark adaptation period	Median b-wave implicit times (ms)	Interquartile range b-wave implicit times	Median b-wave amplitude (μV)	Interquartile range b-wave amplitude (μV)
DA1 (0 min)	41.5*	29.7 – 47.7	14.2*	5.7 – 36.4
DA2 (4 min)	54.0	43.8 – 59.5	29.5*	11.8 – 72.4
DA3 (8 min)	55.6	48.5 – 60.3	54.9	26.1 – 90.9
DA4 (12 min)	54.0	48.1 – 60.3	67.0	37.0 – 104.5
DA5 (16 min)	53.2	47.7 – 57.9	78.4	44.5 – 113.0
DA6 (20 min)	51.7	47.0 – 57.9	78.4	49.0 – 125.0