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Influence of recording electrode type and reference electrode position on the canine electroretinogram

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Abstract

Electroretinography is commonly used to assess the functional integrity of the retina. There are many external variables that can influence the electroretinographic waveforms recorded, and it is important to be aware of these so as not to misinterpret their effects as abnormalities in retinal function. In this study we examined the effect of three different recording electrodes on the ERGs recorded from normal dogs. A bipolar Burian–Allen lens, a monopolar Dawson Trick Litzkow (DTL) fiber electrode, and a monopolar ERG-Jet lens electrode were compared. The effect of altering the distance of the reference electrode from the eye was also examined; using the ERG-Jet lens electrode, the ERG was recorded with the reference electrode placed over the zygomatic arch at 1, 3 and 5 cm caudal to the lateral canthus. The ERGs recorded with the bipolar Burian–Allen lens had significantly lower amplitudes, higher a-wave thresholds and a shallower initial a-wave slope, than those recorded by the two monopolar electrodes. Positioning the reference electrode further from the eye resulted in significantly higher amplitudes. Naka-Rushton fitting and calculation of retinal sensitivity (K) gave significantly different results between the Burian–Allen lens and ERG-Jet lens electrode with the reference electrode 5 cm from the lateral canthus. These results demonstrate that recording electrode type and distance of the reference electrode from the eye significantly affect the ERG tracings of the dog, and may alter the assessment of retinal function that can therefore be derived. Results obtained using these three different types of electrodes cannot be directly compared.

Introduction and background

The electrical changes that occur in the retina in response to light can be recorded at the corneal surface by electrodes, and the resulting waveform is known as the electroretinogram (ERG). Typically, when retinal function is affected by pathological processes, the light-induced electrical activity, and thus the ERG, deteriorates. In a number of retinal diseases, a reduction in ERG amplitudes may be detected prior to ophthalmoscopic changes. The ERG of dogs are investigated in veterinary medicine for a number of different reasons including: the early diagnosis of retinal dystrophies, such as progressive retinal atrophy (PRA) [1]; to differentiate between retinal causes of sudden vision loss, such as sudden acquired retinal degeneration syndrome [2] and central causes [3]; as well as being a useful screening tool for dogs prior to cataract surgery to ensure that underlying retinal disease is not present [4]. The dog with its relative abundance of different

naturally occurring retinal dystrophies is becoming more established as a model for human disease and a species for investigation of therapeutic modalities [5–8]. Because of the increasing use of dog models a good understanding of the canine ERG and factors that influence the tracings obtained is now even more important.

There are many variables that can influence the ERG recordings and the effect of these may be misinterpreted as changes caused by retinal disease. There are two main categories of variables; physiological and instrument-related factors. Physiological factors include breed of dog [1], age [9] and individual variation. Additionally, factors such as diurnal variation [10] and body temperature [11], that have been shown to alter ERG response in other species will most likely also affect ERG tracings in the dog. Other physiological variables are those introduced by the protocol such as degree of pupillary dilation [12], prior exposure to bright light [13], anesthetic agent used [14, 15], and depth of anesthesia [16]. Instrument-related factors that can affect the ERG waveforms consist of variations in the stimulating and recording equipment including discrepancies that can arise from the use of different electrodes [17–26]. There are several types of ERG recording electrodes. They include those that have a contact lens electrode such as the Burian–Allen lens [27] and the ERG-Jet lens [28]. Some contact lens electrodes, including the Burian–Allen as modified by Lawwill and Burian [29] are available as bipolar electrodes. Apart from contact lens electrodes a number of fiber, wick, foil and needle electrodes have been used [22, 24, 30]. A commonly used example of such an electrode is the Dawson Trick Litzkow (DTL) fiber electrode [31].

The influence of electrode type on the ERG recorded has been investigated in some species, including mouse, rat, and human, but only to a limited extent in the dog. Steiss and colleagues [22] reported a comparison of a contact lens electrode and needle electrode that was either placed in the upper eyelid or under the bulbar conjunctiva in the dog. They found higher b-wave amplitudes from the contact lens than the needle electrode and from scrutiny of the figures in their paper the signal to noise ratio is clearly much better in the recording from the contact lens electrode. In a study using both C57BL/6 mice and Wistar strain of rats, the potentials recorded with contact lens electrodes showed significantly larger amplitudes, greater oscillatory potentials, and better reproducibility than those recorded from use of either a cotton-wick silver–silver chloride electrode or a coiled stainless steel wire electrode [17]. A study in humans comparing the ERG recorded by DTL fiber and ERG-Jet lens electrodes found that under scotopic conditions the ERG-Jet lens electrode recorded a significantly larger b-wave in response to brighter flashes. Furthermore the recordings from the DTL fiber electrode showed greater variability than those from the ERG-Jet lens electrode. The authors of the study concluded that the two electrodes could not be used interchangeably [18]. Two separate studies that included a comparison of recordings using the bipolar Burian–Allen lens with those with the DTL fiber in human subjects showed that the DTL fibers gave lower amplitudes [19, 20]. Another study in humans that compared 6 electrodes showed that the Burian–Allen lens gave the highest amplitude responses followed by the ERG-Jet lens then the DTL fiber [21].

The purpose of the study reported here was to compare the ERG recorded by different electrodes in dogs using a commonly utilized veterinary ERG recording device, the Retinographics v5.50 ERG system (RetinoGraphics Inc., Norwalk, CT). We selected three electrodes that are commonly used in dogs for comparison: bipolar Burian–Allen lens [27, 29], DTL fiber electrode [31], and ERG-Jet lens electrode [28]. Additionally, we investigated the effect of altering the distance of the reference electrode from the eye on the recording obtained using the ERG-Jet lens electrode.

Subjects and methods

Subjects

Six crossbred, young adult (median age 2 years), sexually intact, female dogs were used in this study. They were housed in indoor kennels and fed an adult maintenance diet dog food (Hill's Science Diet Adult Original, Topeka, KS). Procedures were approved by the Institutional Animal Care and Use Committee and conformed to the

ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Anesthesia

Animals were premedicated with acepromazine maleate (Butler Company, Columbus, OH) intramuscularly (0.05 mg/kg). Induction of anesthesia was with intravenous thiopental sodium (Pentothal. Abbot Laboratories, North Chicago, IL) (6–8 mg/kg) and maintained with 2.5% isoflurane (IsoFlo. Abbot Laboratories, North Chicago, IL) delivered in oxygen. Body temperature was maintained with a heating pad. Depth of anesthesia was kept constant during the procedure and a pulse-oximeter (Vet/Ox 4400, Heska Inc., Fort Collins, CO) was used to record pulse rate and the oxygen saturation for the duration of the recording.

Preparation for ERG

Prior to anesthesia, the pupil of the left eye was dilated in each dog with 1% tropicamide (Mydriacyl, Alcon Laboratories, Fort Worth, TX) and 10% phenylephrine (AK-Dilate. Akorn Inc., Buffalo Grove, IL). Pupil diameter was examined before and after the procedure with the aid of a dim red light to ensure that maximal pupillary dilation had been achieved and maintained. Eyes were positioned in primary gaze using stay sutures through the bulbar conjunctiva adjacent to the limbus medially, laterally, and dorsally. These stay sutures were then secured using hemostatic clamps to fix the suture in position by clamping the suture to the fur. Different recording electrodes were placed on the cornea with 2.5% methylcellulose (Goniosol, Ciba Vision Ophthalmics, Duluth, GA) used as a coupling agent. The electrodes used were the DTL fiber electrode, the ERG-Jet Disposable Contact Lens, or a bipolar Burian–Allen lens (Figure 1). When using the DTL fiber electrode a reference platinum needle electrode was placed 1 cm posterior to the lateral canthus over the zygomatic arch. When using the ERG-Jet lens the reference needle electrode was placed at different distances from the eye; either 1, 3, or 5 cm posterior to lateral canthus over the zygomatic arch. In each case the ground electrode platinum needle was placed subcutaneously in the dorsal cervical region. Each subject underwent a dark adaptation period of 20 minutes.

Recording of the electroretinogram

A RetinoGraphics (Norwalk, CT) ERG system with a BPM-100 LED strobe light fixed 7.5 cm from the cornea directed along the visual axis was used. The BPM-100 system is commonly used for clinical ERGs by veterinary ophthalmologists. It consists of software that can be run on a PC and hardware consisting of amplifiers and filters with a fixed 6 dB/octave high-pass filter at 0.3 Hz and a 48 dB/octave Bessel low-pass filter at 560 Hz. The computer allows storage and averaging of individual waveforms. There are a choice of stimuli, providing different colored flashes, available for the system. Each consists of an array of 7 LEDs in a 2.5 cm diameter housing.

For this study a dark-adapted intensity series ERG was recorded using brief flashes of light from a white LED array (output 500–640 nm with dominant wavelength at 464 nm, color temperature 6500 K) of the following intensities −2.6, −2.18, −1.59, −1.01, −0.63, 0.00, 0.39, 0.86 log cdS/m^2. Flash intensity was measured using a Research Radiometer IL 1700 with

Figure 1. The ERG electrodes that were used in this study positioned in the eye prior to the ERG procedure. (a) Dawson, Trick, and Litzkow (DTL) fiber electrode (monopolar electrode); (b) ERG-JET lens (monopolar electrode) and (c) Burian–Allen lens (bipolar electrode). Note the conjunctival stay sutures used to keep the eyelids open and to maintain the globe in primary gaze.

SED033 silicon light detector (International Light Inc., Newburyport, MA). Sufficient time was allowed between flashes to prevent light adaptation of the retina (up to 50 seconds for the highest intensity). Between 3 and 5 flashes were averaged at each intensity. The ERG was recorded from the left eye of each dog using each electrode under a single anesthetic procedure. The order in which the recording electrodes were used was different for each dog as shown in Table 1. Subsequent electrodes were placed under dim red light illumination after which 5 minutes of further dark adaptation was allowed.

Data analysis

The amplitudes and implicit times of the a- and b-waves of all ERG tracings obtained in the study were measured and compared. The a-wave amplitude was measured from baseline to peak trough of the a-wave and the b-wave amplitude from peak a-wave trough to peak of the b-wave (not including oscillatory potentials). The a-wave implicit time was measured from flash onset to peak a-wave amplitude, and b-wave implicit time from flash onset to the time of peak b-wave amplitude. The ERG tracing results obtained with the various electrodes and reference electrode distances were compiled and analyzed statistically using repeated measures (ANOVA). If any statistically significant difference was found the data were further analyzed (P values adjusted) using *post hoc* comparisons with Fisher's or Tukey–Kramer tests. Data were deemed significant when $P < 0.05$. This analysis was performed using statistics computer software (StatView, SAS Institute Inc., Cary, NC, USA).

A function relating b-wave amplitude to stimulus luminance was generated using non-linear regression with the 3-parameter Hill equation (Naka-Rushton function) for each test subject [32]. The 3 independent parameters are: the maximum response (V_{bmax}), slope (n) and half-saturation constant (K). This function is as follows:

$$V = (V_{\mathrm{bmax}} \times I^n)/(I^n + K^n),$$

where V denotes the b-wave amplitude for a given flash intensity I; V_{bmax} is the upper asymptote of the amplitude versus intensity function; K is the flash intensity yielding a response amplitude of $1/2V_{\mathrm{bmax}}$; and n is an exponent affecting the slope. K is often considered to reflect retinal sensitivity since it represents a constant criterion ($1/2V_{\mathrm{bmax}}$) that determines where the amplitude versus intensity function is located along the flash intensity axis [33]. The curves were fit using SigmaPlot 2001, version 7.101 (SPSS, Inc), which employs a Marquardt–Levenberg algorithm to perform least-squares fits.

The mean flash intensity value needed to elicit a fixed value of a-wave amplitude (10μV criterion response) and of b-wave amplitude (20μV criterion response) were analyzed and compared for each electrode used in the study.

Results

A- and b-wave intensity:response plots for the different electrode types and the ERG-Jet lens

Table 1. Order of electrodes used in each of the 6 dogs tested

Electrodes	Burian–Allen	ERG-Jet Lens Electrode (distance of reference electrode from canthus)			DTL
		(1 cm)	(3 cm)	(5 cm)	
Animals					
Dog 1	1st	2nd	3rd	4th	5th
Dog 2	2nd	3rd	4th	5th	1st
Dog 3	5th	2nd	3rd	4th	1st
Dog 4	5th	1st	2nd	3rd	4th
Dog 5	1st	2nd	3rd	4th	5th
Dog 6	2nd	3rd	4th	5th	1st

with the reference electrode at different distances from the lateral canthus are shown in Figure 2.

Comparison of ERGs recorded by Burian–Allen lens, ERG-Jet lens and DTL fiber with reference electrodes at 1 cm from canthus (Figures 2(a, c) and 3)

A-wave amplitudes (Figure 2a)

The mean overall a-wave ERG amplitudes recorded with the bipolar Burian–Allen lens were significantly lower than those recorded by the ERG-Jet lens and DTL fiber ($P = 0.0022$ and $P = 0.0182$, respectively). The difference between the ERG-Jet lens (reference electrode 1 cm from the canthus) and the DTL fiber was not significant. The mean a-wave amplitude recorded by the Burian–Allen lens was significantly lower than both the DTL fiber and ERG-Jet lens electrode at the highest light intensity and significantly lower than just the ERG-Jet lens electrode at the second to highest light intensity.

B-wave amplitudes (Figure 2c)

Mean overall b-wave ERG amplitudes recorded with the bipolar Burian–Allen lens were significantly lower than those recorded by the ERG-Jet lens and DTL fiber ($P < 0.0001$ and $P = 0.0011$, respectively). The mean b-wave amplitude recorded using the Burian–Allen lens was lower than both the ERG-Jet lens and DTL fiber at the brightest 5 flash intensities. There was no statistically significant difference in the overall b-wave amplitude between the ERG-Jet lens electrode and the DTL fiber electrode ($P = 0.3984$).

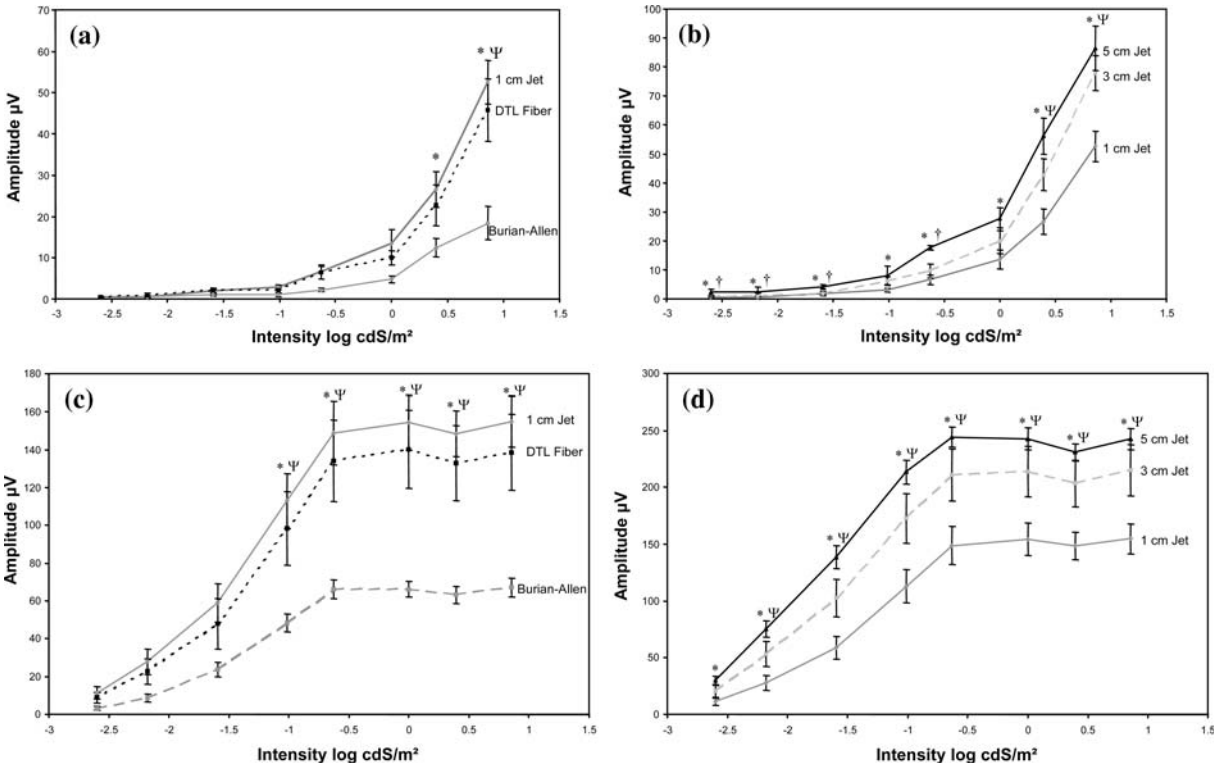

Figure 2. Intensity:response plots for mean (±SEM) a-wave (a and b) and b-waves (c and d). a and c are a comparison between bipolar Burian–Allen lens, DTL fiber electrode and ERG-Jet electrode with the reference electrode 1 cm from the lateral cantus. b and d are a comparison between ERG-Jet electrode with the reference electrode 1, 3 or 5 cm from the lateral canthus. Key: (a). * indicates a significant difference between ERG-Jet lens and Burian–Allen lens. Ψ indicates a significant difference between DTL fiber and Burian–Allen lens. (b). * indicates a significant difference between reference electrode at 1 cm and 5 cm. Ψ indicates a significant difference between reference electrode at 1 cm and 3 cm.† indicates a significant difference between reference electrode at 3 cm and 5 cm. (c) * indicates a significant difference between ERG-Jet lens and Burian–Allen lens. Ψ indicates a significant difference between DTL fiber and Burian–Allen lens. (d) * indicates a significant difference between reference electrode at 1 cm and 5 cm. Ψ indicates a significant difference between reference electrode at 1 cm and 3 cm.

Implicit times (Figure 3)
There was no overall difference between the electrodes when the implicit times of the a- and b-waves were compared (data not shown). However, when the mean a-wave implicit time for each flash light intensity was analyzed separately the implicit time recorded with the Burian–Allen electrode was significantly greater than that for the ERG-Jet lens and DTL fiber electrodes at two of the 8 flash intensities (Figure 3).

Comparison of reference electrode position (Figure 2(b, d))

A-wave amplitude (Figure 2b)
Positioning the reference electrode further caudally from the eye (5 > 3 > 1 cm) tended to result in significantly greater a-wave amplitudes ($P < 0.05$). The results with the 3 cm electrode position were significantly lower than the 5 cm position at the lower flash intensities only (at 4 of the 5 lowest intensities). The 5 cm electrode amplitudes were significantly greater than those of the 1 cm electrode at all intensities. The 3 cm electrode amplitudes were significantly greater than those of the 1 cm electrode in response to the brightest two flash intensities (Figure 2b).

B-wave amplitude (Figure 2d)
Positioning the reference electrode at 3 and 5 cm from the lateral canthus resulted in significantly greater mean b-wave amplitudes compared to the amplitude obtained with the reference electrode at 1 cm from the canthus ($P < 0.001$). When the results at individual flash intensities were analyzed the mean b-wave amplitude recorded with the reference electrode 5 cm from the canthus was significantly greater than the results when the electrode was 1 cm from the canthus for each flash intensity. The mean b-wave amplitude with the reference electrode 3 cm from the canthus was significantly greater than when the electrode was 1 cm from the canthus at all but the lowest flash intensity. There was no significant difference, however, in the comparison between the b-wave amplitudes obtained with the reference electrode placed at 3 and 5 cm from the lateral canthus ($P = 0.0670$) (Figure 2d).

Slope of the a-wave
The ERG tracings resulting from a flash of 0.9 log cdS/m^2 recorded with the different electrodes were normalized in relation to their a-wave amplitudes and then plotted together in order to enable a comparison of the initial slope of the

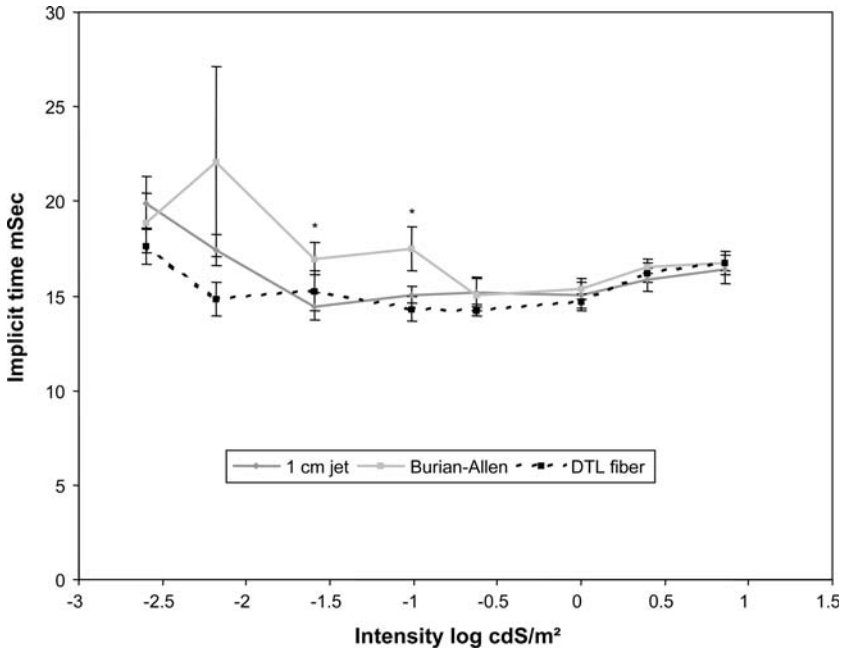

Figure 3. Mean (±SEM) a-wave implicit time for Burian–Allen lens, DTL fiber and ERG-Jet lens. * indicates a statistical difference between the recordings from the Burian–Allen lens and ERG-Jet lens with reference electrode placed 1 cm caudal to the lateral canthus.

a-waves to be made. The normalized overlapping ERG tracings demonstrated that the a-wave obtained with the Burian–Allen lens was less steep compared to the ones obtained with the monopolar electrodes. The slope of the a-wave obtained with the ERG-Jet lens with the reference electrode at different distances from the canthus very closely overlapped after normalization (See Figure 4).

A-wave threshold

Analyzing the raw ERG results (detailed intensity: response plots), obtained with the different lenses used in the experiment, differences in a-wave thresholds were observed. The 10μV criterion mean a-wave threshold for the Burian–Allen lens (0.45 ± 0.26 log cdS/m^2) was significantly higher ($P < 0.05$) than the other electrodes or lenses – DTL fiber (-0.36 ± 0.26 log cdS/m^2), ERG-Jet lens at 1 cm (-0.44 ± 0.56 log cdS/m^2), 3 cm (-0.49 ± 0.43 log cdS/m^2) and 5 cm from canthus (-0.9 ± 0.32 log cdS/m^2).

B-wave threshold

A difference in the 20μV criterion mean b-wave thresholds also was observed when comparing the Burian–Allen lens with the ERG-Jet lens electrode. The threshold for the Burian–Allen lens (-1.69 ± 0.26 log cdS/m^2) was significantly higher ($P < 0.05$) than for ERG-Jet lens at 1, 3 and 5 cm from canthus (-2.25 ± 0.41, -2.54 ± 0.35, -2.71 ± 0.24 log cdS/m^2, respectively) and DTL fiber ($-2{,}19 \pm 0.53$ log cdS/m^2).

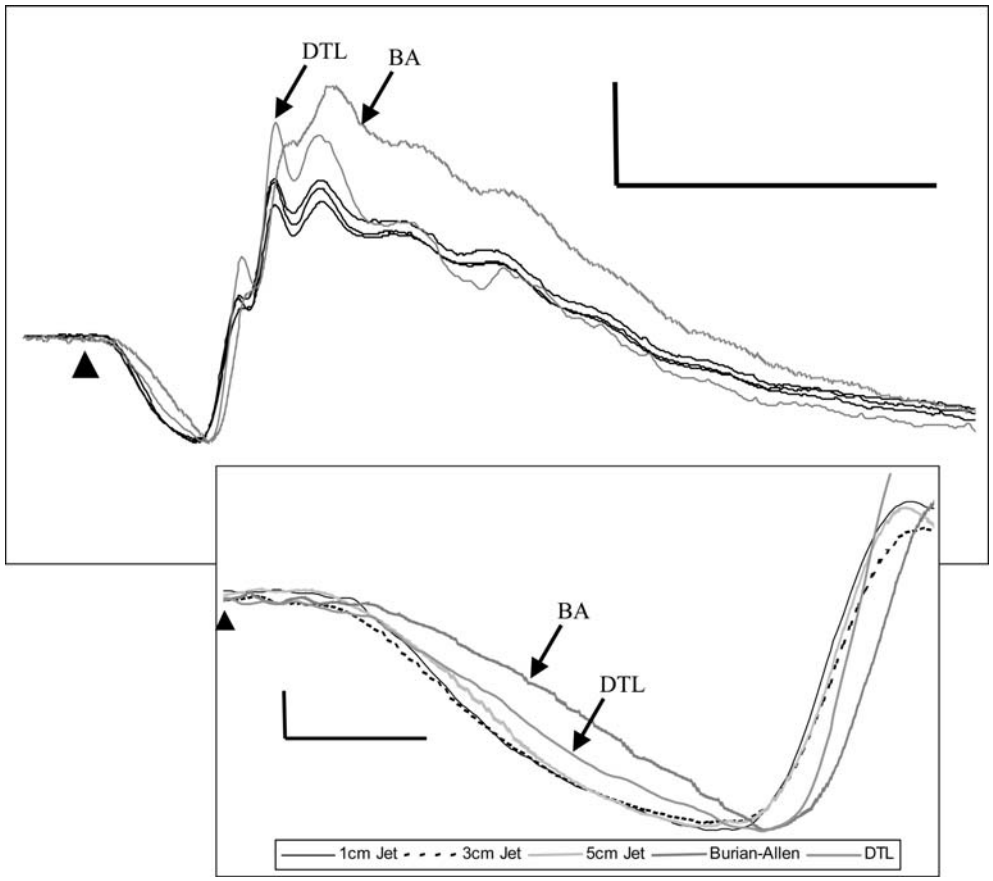

Figure 4. Representative normalized overlapping tracings from one dog resulting from a 0.9 log cdS/m^2 flash stimulus for all electrodes. The insert shows a more detailed view of the initial slope of the a-wave. Note that the slope and shape of the a-wave obtained with the Burian–Allen electrode (black arrow) is less steep compared to the ones obtained with the monopolar electrodes. Also note that even though the ERG-Jet lens with the reference electrode at different distances from the canthus produced waveforms of different amplitudes, after normalization these same tracings almost perfectly overlap. Arrowhead indicates flash. In the main figure the vertical bar= 100μV; Horizontal bar = 50 mSec. In the insert the vertical bar= 20μV; Horizontal bar = 5 mSec. (note that the scales for amplitudes are only relevant to the waveform from the 5-cm Jet lens as the other waveforms were normalized to the a-wave amplitude recorded by the 5-cm Jet lens).

Table 2. Mean V_{bmax} results obtained following Naka-Rushton fitting of individual b-wave intensity:response curves for each of the electrodes used in the experiment

Electrode Type (Reference electrode distance from canthus)	Number of dogs	Mean V_{bmax} μ Volts	Standard Deviation	Standard Error
Jet Lens (1 cm)	6	156.6	32.8	13.4
Jet Lens (3 cm)	6	216.4	54.8	22.4
Jet Lens (5 cm)	4	245.4	18.6	9.3
Burian–Allen (n/a)	6	68.2	9.5	3.9
DTL (1 cm)	6	158.9	60.1	24.5

Naka-Rushton fitting results

The Naka-Rushton equation was fitted for each individual b-wave intensity:response series of every dog. The resulting R values were between 0.76 and 0.97 and R^2 values between 0.7 and 0.96, indicating that the data successfully fitted the equation.

V_{bmax} values (Table 2) corresponded to the mean maximal amplitudes of the b-wave at the first limb of the intensity:response plot. V_{bmax} was significantly lower when the Burian–Allen electrode was used. When the ERG-Jet lens was used, the V_{bmax} was also significantly lower when the reference electrode was placed 1 cm caudal to the lateral canthus compared to 3 or 5 cm from the lateral canthus. See Table 2 for the mean V_{bmax} value of every electrode used and Table 3 for the significance (P values) when comparing the V_{bmax} results obtained these different electrodes.

The K parameter (the intensity required to elicit a b-wave of $1/2V_{bmax}$) is regarded as a parameter for retinal sensitivity. K was significantly different only when the results obtained with Burian–Allen electrode (-1.32 ± 0.06 log cdS/m^2) were compared with the ones obtained with the ERG-Jet lens with the reference electrode placed at 5 cm from the lateral canthus (-1.75 ± 0.7 log cdS/m^2, $P = 0.048$) (See Figure 5). No difference was observed when the n value (slope) was analyzed (data not shown).

Discussion

Differences in ERG recording protocols can make it difficult to directly compare results from different laboratories. The results of this study show that the recording electrode used can have a significant effect on the ERG tracings recorded from dogs, and also on the assessment of retinal function that can be derived from those recordings.

The fact that there was a significant difference in ERG amplitudes recorded with different electrodes in the dog although important to record, was not surprising in view of similar findings in other species [17–21, 34, 35]. Interestingly, in the dog we found that the results using the different electrode types differed from those previously reported for human subjects. In the dog consistently larger amplitudes are seen with both the ERG-Jet lens and DTL fiber electrodes compared to the Burian–Allen lens, whereas in human subjects the Burian–Allen lens is reported to record greater amplitudes than either the ERG-Jet lens or the DTL fiber [19–21]. The differences observed between our results and those in human

Table 3. Significance (P value < 0.05 indicated by *) for the comparison of the mean V_{bmax} results between electrodes used in the experiment.

Electrode Comparison	P-value
Jet (1 cm) vs. Jet (3 cm)	0.028*
Jet (1 cm) vs. Jet (5 cm)	0.031*
Jet (1 cm) vs. Burian–Allen	0.013*
Jet (1 cm) vs. DTL	0.9245
Jet (3 cm) vs. Jet (5 cm)	0.2921
Jet (3 cm) vs. Burian–Allen	< 0.0001*
Jet (3 cm) vs. DTL	0.0256*
Jet (5 cm) vs. Burian–Allen	< 0.0001*
Jet (5 cm) vs. DTL	0.0039*
Burian–Allen vs. DTL	0.001*

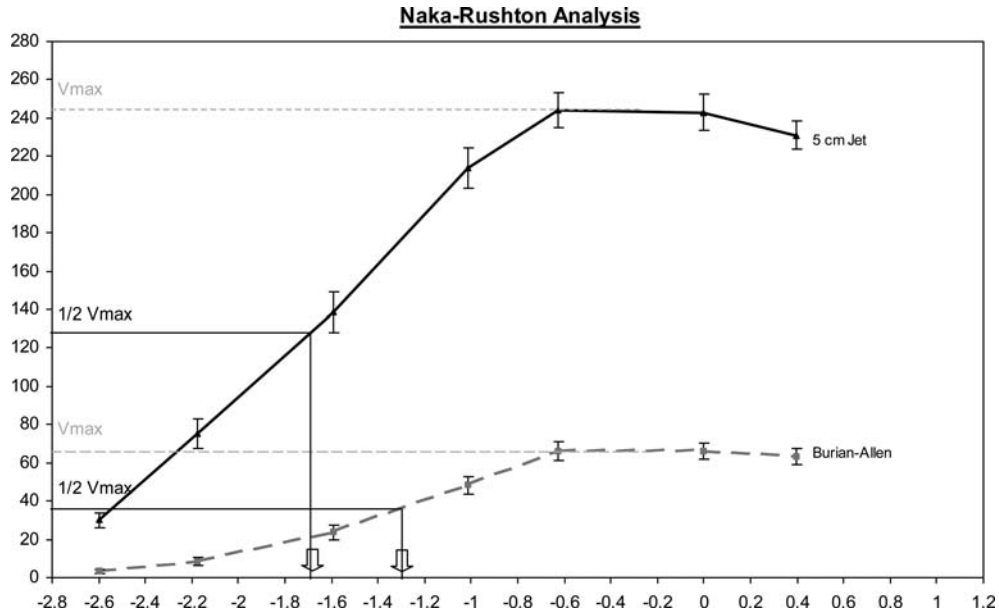

Figure 5. Naka-Rushton fit parameters. Mean scotopic b-wave intensity:response function obtained with the ERG-JET lens with the reference electrode placed at 5 cm from the lateral canthus (5 cm Jet) (black solid line) and the one obtained with the Burian–Allen lens (gray dashed line). V_{bmax} = maximal rod b-wave amplitude of the first limb of the response curve (dashed line); K = log intensity at half maximal rod response ($1/2V_{bmax}$) (open arrows).

subjects may be due to differences in anatomy between the human and dog globe and orbit as discussed later.

The finding with the ERG-Jet lens electrode that moving the reference electrode further caudally resulted in higher amplitudes may explain the relatively low amplitudes observed with the Burian–Allen electrode where the built in reference electrode on the eyelid speculum is sampling electrical fields from the palpebral conjunctiva and is thus in a similar plane to the corneal electrode that is built in to the Burian–Allen contact lens. A study recording the ERG response from a perfused dog eye using a corneal contact lens referenced to a scleral electrode at a variable distance caudal from the limbus found that the b-wave amplitude was low when the reference electrode was at the limbus, but rapidly increased as the reference electrode was moved 5–6 mm caudal to the limbus to reach a maximum at the point that the optic nerve exited the globe [36]. This coupled with the fact that unlike the human the dog does not have a complete bony orbit, means that a skin electrode placed over the zygomatic arch in the dog has predominantly soft tissues in a direct line between the reference electrode and the posterior sclera. Soft tissues have much lower electrical resistance than bone [37]. The complete bony orbit of the human would mean that currents from the posterior sclera would predominantly flow anteriorly through soft tissues to exit the orbit and thus reach skin electrodes, where in the dog they could presumably exit the orbit through the soft tissues that make up the majority of the lateral wall of the orbit allowing greater conductance of currents from the posterior of the globe to a more posteriorly positioned electrode than would occur in a similarly positioned electrode in man. The combination of a contact lens electrode with a reference electrode 5 cm caudal to the lateral canthus would sample the electrical field across the globe in an approximate anterior–posterior direction and would thus be expected to record greater amplitudes than when the reference electrode samples in a plane over the equator of the eye (e.g. the position 1 cm caudal to the lateral canthus).

Analyzing the b-wave intensity:response function using the Naka Rushton formula allows for an estimate of retinal homogeneity and retinal sensitivity. In this study we found that the measure of retinal sensitivity (log scotopic intensity

that produces a b-wave amplitude of $1/2V_{bmax}$, K) was significantly different between the ERG-Jet electrode with a reference electrode 5 cm caudal to the lateral canthus and the Burian–Allen bipolar lens. As well as differences in amplitudes, K value and a- and b-wave thresholds between the ERG recorded with the Burian–Allen bipolar lens and the ERG-Jet lens and DTL fiber electrodes the slope of the a-wave was also different for the Burian–Allen lens recordings. The initial slope of the scotopic a-wave is predominantly derived from photoreceptor responses and appeared different between the recording lenses. Normalizing the peak a-wave response following subtraction of intensity matched light adapted responses and comparing the slopes is suggested by Hood and Birch [38] as a simplified method of assessing photoreceptor sensitivity and maximal response. The suggested method could not be utilized in this study because of limitations in flash intensity and because photopic ERGs were not recorded. The more complex method of performing a-wave modeling was also not performed in this study [39]. Further studies utilizing a-wave modeling would be interesting to see if the calculated PIII response differed depending on which recording electrode was used.

Previous studies with the DTL-fiber electrode have shown the importance of position of the fiber on the eye. In human subjects there can be a substantial variation in amplitude recorded with the DTL fiber depending whether it is lying across the center of the cornea or is positioned within the conjunctival sac [40]. Although similar studies are not reported for the dog, and we were careful to keep the fiber across the center of the cornea, there may be similar variation if the fiber is not consistently positioned on the cornea in the same manner for each ERG.

The ERG stimulus used in this study was not a Ganzfeld stimulus as is usually recommended [41, 42]. We chose to use this particular ERG stimulus and recording equipment for this study because of its wide use by veterinary ophthalmologists. The lack of Ganzfeld stimulation means that a homogenous stimulation of the entire retina was not achieved. This would also mean that if a contact lens electrode reduced the effective pupil aperture it might have a more profound effect on retinal stimulation than if a Ganzfeld stimulus had been used [43]. This would apply to the Burian–Allen lens if the opaque speculum obscured part of the pupil. However with the size of the Burian–Allen lens used the pupil aperture would not have been reduced.

Although selecting an electrode that results in higher recorded ERG amplitudes may seem desirable, as any reduction in amplitudes due to early stages of a disease causing retinal dysfunction/degeneration may be more apparent when all amplitudes are relatively larger, other factors need also to be considered in the selection of a recording electrode. These include the stability of the baseline, signal to noise ratio and variability of recordings. Although we made no attempt to quantify these factors during this study, it is our subjective assessment that the bipolar Burian–Allen lens gives a more stable baseline than the other electrodes and a better signal to noise ratio.

In conclusion this study very clearly demonstrates that to allow a comparison between ERG tracings recorded from individual dogs the recording electrode and position of the reference electrode must be standardized, as must the many other factors that can alter the ERG recorded.

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