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Electroretinogram responses of the normal thoroughbred horse sedated with detomidine hydrochloride

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Abstract

Purpose The main objective was to record electroretinogram (ERG) parameters of normal thoroughbred mares using the HMsERG, a mini-Ganzfeld electroretinographic unit, and a contact lens electrode. The second objective was to determine whether IV detomidine hydrochloride at 0.015 mg/kg is consistently an effective choice for sedation of horses undergoing this ERG protocol.

Methods The study population consisted of 30 normal thoroughbred mares. ERG data were harvested using a protocol that included three different light intensities (10, 3000, and 10 000 mcd s/m^2) and a 30-Hz flicker at 3000 mcd s/m^2.

Results Mean, median, standard deviation, and estimated normal ranges using the 5–95% of the data for a- and b-wave implicit times (IT), amplitudes (AMP), and b/a ratios were reported. Scotopic results at low intensity (10 mcd s/m^2) had estimated ranges for b-wave IT of 41.8–72.9 ms and AMP of 19.8–173.3 μV. Middle intensity (3000 mcd s/m^2) a-wave IT was 13.2–14.7 ms with a-wave AMP of 68.4–144 μV; the b-wave IT was 28.7–41.5 ms with b-wave AMP of 105.7–271.5 μV; and the b/a ratio was 0.95–2.71. The high-intensity (10 000 mcd s/m^2) average recordings showed an a-wave IT of 13–14.9 ms, a-wave AMP of 85.7–186.8 μV; b-wave IT of 26.6–45.4 ms, b-wave AMP of 104.7–250.6 μV; and a b/a wave ratio of 0.7–2.0. The 30-Hz cone flicker showed an IT of 22.8–28.9 ms and AMP of 44.1–117.1 μV.

Conclusions Results of normal thoroughbred ERG responses are reported. The protocol proved to be simple and safe and provided consistent results.

Key Words: electroretinogram, HMsERG, contact lens electrode, detomidine hydrochloride, equine, thoroughbred horse

INTRODUCTION

Electroretinography (ERG) is the noninvasive method of choice used to objectively assess retinal function in veterinary ophthalmology.[1] Full-field flash ERG assesses photoreceptor-mediated responses from the entire retina.[1–4] ERG studies evaluate gross retinal function, and characterize and diagnose retinopathies and inherited photoreceptor dystrophies.[1,5–9] By altering the light stimulus intensity delivered to the eye, following periods of dark and/or light adaptation, different photoreceptor subpopulations can be preferentially stimulated.[1–3,5]

The International Society for Clinical Electrophysiology of Vision (ISCEV) developed and recommends a standardization of ERG protocols for human patients to allow for comparison between different institutions.[3,4] Using this protocol, similar guidelines for clinical ERG in the dog have been published, to assist with the diagnosis of photoreceptor disorders.[1] However, few ERG studies have been reported in the equine species. Using a modification of the published dog ERG protocol, Komáromy *et al.*[7] assessed flash ERG responses in two breeds of horses using a DTL microfiber electrode and a pseudo-Ganzfeld recording device. Full-field Ganzfeld stimulation is recommended when recording ERGs to acquire homogenous retinal activity with minimal light scattering.[1,3,4,10] Recently, characterization of the normal dark adaptation curve of the horse was reported using a mini full-field Ganzfeld device and a contact lens electrode.[2] The results indicate that full-field flash ERG in the horse should be performed following a minimum of 20 min of dark adaptation.[2] To date, expected normal limits have not been reported for horse ERGs using mini-Ganzfeld stimulation and a contact lens recording electrode.

Electroretinography in horses appears to be underutilized in the clinical setting probably because it is perceived to be

technically challenging, inaccurate, and unsafe.[2,7,11] It has been suggested that appropriate sedation should be deeper than used for routine examination to minimize both head movement and noise originating from extraocular muscle movement.[2] Sedation protocols, using detomidine hydrochloride, have been reported to be adequate for obtaining clinical ERGs in the equine.[2,7,9,12] Sandmeyer *et al.*[9] utilized an indwelling intravenous catheter in the jugular vein to sedate horses with a bolus of 0.01 mg/kg detomidine hydrochloride followed by a continuous drip of 12 mg detomidine/500 mL saline at a rate of 1 drop per second. Other studies have reported the sole use of a one-time dose of IV detomidine hydrochloride at 0.015 mg/kg.[2,7]

Detomidine hydrochloride is a highly selective α2-adrenergic agonist that causes analgesia, sedation, and muscle relaxation in the horse. It is rapidly distributed and has a reported half life of ~30 min.[13,14] Side effects of IV detomidine hydrochloride in the horse include an increase in the duration of systole, diastole, or both, and decreases in heart rate, blood volume, and blood flow velocity within the internal iliac artery.[15,16] There are no known adverse effects with local vascular perfusion within the ovaries and endometrium of horses, confirming the safety of IV use in pregnant and nonpregnant mares.[15,17] Additionally, IV detomidine hydrochloride in the horse has been shown to cause a decrease in intraocular pressure (IOP) at 10 and 20 min postadministration.[16]

The objective of this study was to record ERG parameters of normal thoroughbred horses sedated with IV detomidine hydrochloride at a dose of 0.015 mg/kg using the Handheld Multispecies ERG (HMsERG) Model 1000 (RetVetCorp Inc., Columbia, MO, USA), with its mini-Ganzfeld half-sphere bowl system, and a gold foil contact lens electrode (ERG-jet™; Fabrinal SA Tuilerie 42, 2300 La Chaux-de-Fonds, Switzerland). A second objective of the study was to assess the efficacy of a bolus dose of IV detomidine hydrochloride in creating adequate sedation to obtain flash and flicker ERGs.

MATERIAL AND METHODS

Animals

The ERG responses were recorded from 30 healthy thoroughbred mares. The horses were found to be healthy on physical examinations and were housed at a privately owned facility in Arizona. Informed consent was obtained from the owner prior to enrollment in the study. All horses had complete ophthalmic examination by the same individual (JN), at least 24 h prior to ERG testing, including Schirmer Tear Test I (STT-1), IOP via applanation tonometry (Tonopen XL, Mentor Inc., Norwell, MA, USA), slit-lamp biomicroscopy, and binocular indirect and direct ophthalmoscopy. Only healthy thoroughbred mares without ophthalmic abnormalities were included. The age and coat color of each horse was recorded.

Preparation, electrode setup, and recording equipment

The body weight of each horse was estimated by the same individual (MC), using an equine measuring tape at the withers. One eye was randomly selected, via coin toss, for ERG testing. Sedation was administered via the jugular vein using a single bolus of 0.015 mg/kg detomidine hydrochloride (Dormosedan¬; Pfizer Animal Health, New York, NY, USA).[2,7] Immediately following detomidine administration, 0.2 mL of 1% tropicamide (Tropicamide; Falcon Pharmaceuticals, Fort Worth, TX, USA) was applied to the selected eye for pharmacologic mydriasis, and dark adaptation was initiated. Approximately 10 min later, with use of a red light, an auriculopalpebral nerve block was performed with 2 mL of a 2% lidocaine hydrochloride (Lidocaine 2%; Hospira Inc., Lake Forest, IL, USA) for orbicularis akinesia. The impedance to ground was measured on each horse before recording was started using the test resistor to ensure the impedance was <5000 Ohms.[1,3,4] After 5–7 min, 0.2 mL of 0.5% proparacaine (Alcon Laboratories, Fort Worth, TX, USA) was applied as an anesthetic to the selected eye. Electrodes were placed at previously described anatomic landmarks[2,7] (Fig. 1). The electrode pod was anchored to the halter using an elastic wrap (CoFlex; Adnover Healthcare Inc., Salisbury, MA, USA). Male platinum subdermal needle electrodes (FD-E2-24; Grass Technologies, Astro-Medical, Inc., West Warwick, RI, USA) were used for the reference and ground electrodes, with the ground electrode placed subdermally over the occipital bone and the reference electrode placed approximately 3 cm caudal to the lateral canthus. A contact lens monopolar electrode (ERG-jet; Nicolet Instruments, Madison, WI, USA) with 2.5% methylcellulose (Gonak™; Akorn, Inc., Buffalo Grove, IL, USA) was applied to the axial cornea (Fig. 2). To assist in stabilization, white porous tape was applied near the ends of all electrodes and anchored to the skin. Prior to recording ERG responses,

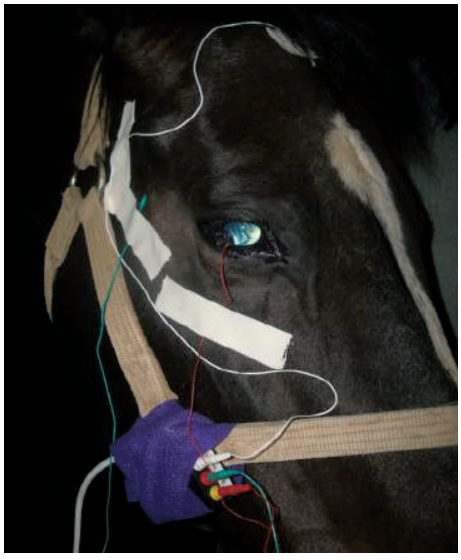

Figure 1. Placement of ERG electrodes.

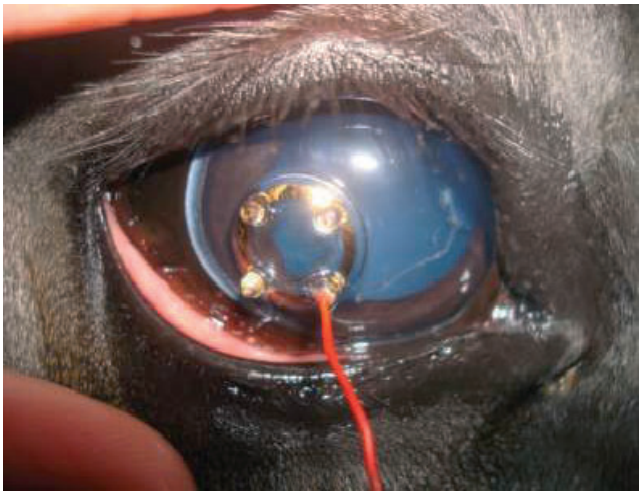

Figure 2. Placement of contact lens electrode.

the electrodes were baseline-tested to ensure that the amplitude (AMP) of the environmental and ambient noise was <30 μV.[1,3,4] The low band-pass limit was 0.3 Hz, and the upper was 300 Hz.[1,3,4] ERG recordings were started following a total of 20 min of dark adaptation.[1,2] During the recording period (Fig. 3), manual blinking was performed to prevent corneal desiccation.

At the end of the ERG recording session, the electrodes were removed, the selected eye was flushed with sterile saline and examined with slit-lamp biomicroscopy, and topical antibiotic ointment (Neomycin/Polymyxin B/Bacitracin; Baush & Lomb Inc., Rochester, NY, USA) was applied to provide corneal lubrication. Horses recovered in individual stalls without access to feed.

Testing protocol

The ISCEV standards were utilized, and preprogrammed custom settings within the HMsERG provided averaging to reduce potential artifact from background noise. The sequence of ERG tests are listed in Table 1. In scotopic settings, rod function was tested by stimulating the dark-adapted retina with low levels of light at 10 mcd s/m^2 with 0.2 s intervals. Following this, mixed rod and cone stimulation was performed at 3000 mcd s/m^2 with 2.5 s intervals and 10 000 mcd s/m^2 with 5 s intervals. Lastly, the horses were slowly introduced to 10 min of intensifying light from the HMsERG unit to record cone stimulation using a 30-Hz flicker response test at 3000 mcd s/m^2. Once all ERG recordings were acquired, the room lights were then turned on and the electrodes were removed from the horse.

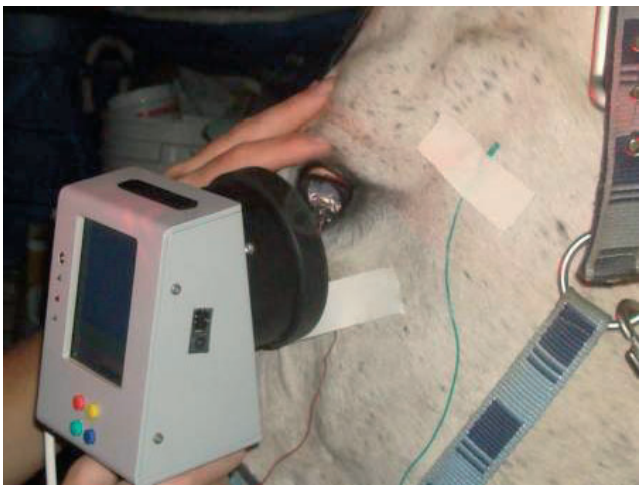

Figure 3. Recording ERG using the HMsERG and a contact lens electrode.

Sedation-related observations

Subjective values were recorded during the ERG protocol to assess the efficacy of the detomidine hydrochloride sedation. The level of sedation as determined by the horse's response or lack of response to stimuli from surrounding noise and movement was recorded and denoted a value from 1 to 5 (excitable = 1, awake = 2, mildly sedated = 3, moderately sedated = 4, and heavily sedated = 5). The head position was recorded with level 1 being above the heart, level 2 at the heart, and level 3 below the heart. Globe position was recorded as centrally positioned or deviated throughout the ERG protocol. All indiscernible poor quality ERG waves and resultant data were not included in the study and accounted for the overall percentage of horses not amenable to collection.

Data analysis

The AMP and implicit time (IT) were measured and reported for each ERG response using the HMsERG Desktop ERG Viewer Application (ERGVIEW). For the pure rod response, only the b-waves were analyzed. For the mixed rod and cone response, a- and b-waves and b/a ratios were calculated. The a-wave AMP was measured from the baseline to the first negative trough, and the b-wave AMP was measured from the trough of the a-wave to the following positive peak of the b-wave. IT was measured as the time between the flash stimulus and the trough or peak of each particular response. The flicker wave AMP was measured from the trough to the following peak and IT from the time of the previous flash to the next positive peak. Descriptive statistical analysis was performed using the SAS v9 software (SAS Institute Inc., Cary, NC, USA). Mean, median, standard deviation, and estimated normal limits for a- and b-wave IT and AMP and b/a ratios were obtained and

Table 1. Sequences of ERG tests

Test #	Response	Intensity (mcd s/m^2)	Flash interval (s)	Adaptation time
1	Rod	10	0.2	20 min, dark
2	Rod and cone	3000	2.5	20 min, dark
3	Rod and cone	10 000	5	20 min, dark
4	Cone (30-Hz flicker)	3000	0.00023	10 min, light

Table 2. Summarized statistical and measured ERG data

Variable	Number (%)	Mean	Median	SD	Min	Max
Low (b-wave IT)	30 (100)	57.4 ms	56.4 ms	9.36 ms	41.4 ms	77.3 ms
Low (b-wave AMP)	30 (100)	96.5 μV	89.00 μV	46.19 μV	34.0 μV	255.0 μV
Mid (a-wave IT)	30 (100)	13.9 ms	14.05 ms	0.44 ms	13.0 ms	14.7 ms
Mid (a-wave AMP)	30 (100)	106.2 μV	106.5 μV	22.75 μV	58.0 μV	153.0 μV
Mid (b-wave IT)	30 (100)	35.1 ms	36.1 ms	3.8 ms	27.7 ms	42.1 ms
Mid (b-wave AMP)	30 (100)	188.6 μV	184.0 μV	49.9 μV	71.0 μV	370.0 μV
High (a-wave IT)	28 (93)	14.0 ms	13.9 ms	0.6 ms	12.7 ms	15.3 ms
High (a-wave AMP)	28 (93)	136.3 μV	137.0 μV	30.4 μV	91.0 μV	217.0 μV
High (b-wave IT)	28 (93)	36.0 ms	36.0 ms	5.7 ms	24.9 ms	45.7 ms
High (b-wave AMP)	28 (93)	177.6 μV	168.5 μV	43.8 μV	75.0 μV	294.0 μV
Flicker IT	27 (90)	25.8 ms	26.0 ms	1.9 ms	19.4 ms	27.8 ms
Flicker AMP	27 (90)	80.6 μV	81.0 μV	21.9 μV	49.0 μV	133.0 μV

AMP, amplitude; IT, implicit time

analyzed. Estimated normal ranges were made by assuming that the responses were normally distributed. The normal ranges were estimated by the 5th and 95th percentiles, or 90% of the total data.[1,3,4] Additionally, a 90% confidence interval was found for both the 5th and 95th percentiles.

RESULTS

Study population

ERGs were performed on 30 thoroughbred horses with a median age of 6 years (range, 1–20 years). Twelve (40%) right eyes and 18 (60%) left eyes were randomly selected and tested. Coat colors were recorded: three were gray (10%), seven were chestnut (13%), and 23 of the horses (77%) were bay. The mean age of the horses tested was 7.3 years with a range of 1–20 years. Using a Kruskal–Wallis test, there was no significant differences within the study population with regards to eye tested, age, and coat color and resultant ERG responses.

Sedation-related observations

Two of the horses (7%) were reported to have minimal sedation, 10 (33%) had mild sedation, and 18 (60%) had a moderate level of sedation throughout the ERG testing. All of the tested globes were centrally positioned. None of the horses were considered to be too heavily sedated or unsedated. Four (13%) of the horses had a head position at the level of the heart, and the remaining horses (87%) had a head position below the heart. Data were analyzed comparing the horses with head positions at and below the level of the heart. Based on the Wilcoxon Rank Sum test, there was a significant difference ($P = 0.0024$) in high light intensity b-wave AMP only. The median value for horses with head positions at the heart was 124 μV compared with 170 μV with head positions below the heart.

The percentage of consistently recorded data was reported and is summarized in Table 2. These results show the number of horses amenable to recording of each of the ERG responses. At low and middle light intensity, 100% of the horses tested had recordable ERG response data. However, with the high light intensity stimulus and cone flicker response test, 93% (28/30) and 90% (27/30) of the horses, respectively, allowed for recordable data without movement. Using a Wilcoxon Rank Sum test, there was no significant difference ($P = 0.1389$) between the level of sedation and amenability to recording ERG responses.

ERG recordings

Figure 4 is an example of the custom ERG waveforms acquired for each horse. The mean, median, standard deviation, minimum, and maximum values for each of these recordings from the 30 horses tested are summarized in Table 2. Estimated normal ranges for each response test are reported in Table 3; further, Table 4 displays each response

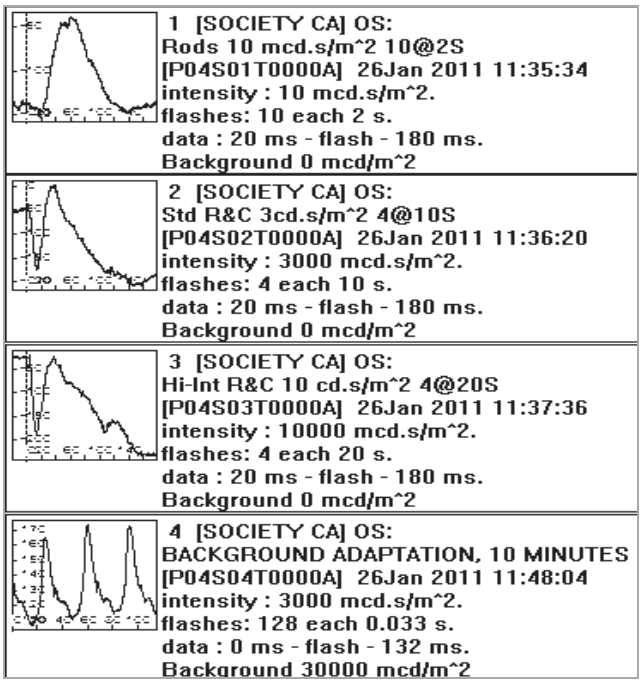

Figure 4. Example of ERG recordings from one horse using International Society for Clinical Electrophysiology of Vision standards.

Table 3. Estimated normal ranges for each ERG response test

Light intensity	a-wave IT (ms)	a-wave AMP (μV)	b-wave IT (ms)	b-wave AMP (μV)	b/a ratio
Low (10 mcd. s/m^2) *Rods	n/a	n/a	41.8–72.9	19.8–173.3	n/a
Middle (3000 mcd. s/m^2) *Rods/cones	13.2–14.7	68.4–144	28.7–41.5	105.7–271.5	0.95–2.71
High (10 000 mcd. s/m^2) *Rods/cones	13–14.9	85.7–186.8	26.6–45.4	104.7–250.6	0.7–2.0
30-Hz Flicker *Cones	n/a	n/a	22.8–28.9	44.1–117.1	n/a

AMP, amplitude; IT, implicit time

Table 4. Number of horses reported for each test, and the mean and standard deviations for the estimated normal ERG data

ERG responses	Number of horses	Mean	Standard deviation
10 mcd. s/m^2			
b-wave IT (ms)	30	57.35	9.36
b-wave AMP (μV)	30	96.53	46.19
3000 mcd. s/m^2			
a-wave IT (ms)	30	13.94	0.44
a-wave AMP (μV)	30	106.20	22.75
b-wave IT (ms)	30	35.13	3.84
b-wave AMP (μV)	30	188.60	49.88
10 000 mcd. s/m^2			
a-wave IT (ms)	28	13.95	0.56
a-wave AMP (μV)	28	136.25	30.39
b-wave IT (ms)	28	36.01	5.66
b-wave AMP (μV)	28	177.64	43.83
30-Hz Flicker			
IT(ms)	27	25.84	1.86
AMP (μV)	27	80.59	21.94

AMP, amplitude; IT, implicit time.

test with the number of horses recorded, mean, and standard deviation. To report normal ERG findings, ISCEV recommends listing median values and the estimated normal ranges using the 5th and 95th percentiles of the data.[1,3] The ERG data recorded were of good quality and easily interpreted, and any indiscernible waves were excluded. At low and middle light intensities, 100% of the horses had recordable data. However, with high light intensity stimulus and flicker response test, 93% and 90% of the horses, respectively, allowed for recordable data without movement. Distinct oscillatory potentials (OPs) were not elicited using this protocol.

DISCUSSION

Electroretinography is used in equine ophthalmology to evaluate the retina when impaired retinal function is suspected or when an anterior segment opacity, such as uveitis, hyphema, or a cataract, prevents fundic evaluation. Thoroughbred horses have been reported to be affected by congenital abnormalities such as microphthalmos, cataracts, and optic disk colobomas.[18–20] In 2006, potential vision-threatening eye disease was reported in 7.4% (15/204) of thoroughbred racehorses from Australia and included cataracts, lens luxation, and large active and inactive chorioretinal lesions.[21] All breeds of horses can be affected with various retinal diseases that would benefit from ERG analysis such as congenital stationary night blindness,[7,9,12] equine recurrent uveitis (ERU),[7,22,23] non-ERU related retinitis and chorioretinitis,[7,24] glaucoma,[7,25] retinal detachment,[7,24,26] ocular trauma.[7,24] Understanding the ERG responses of the normal thoroughbred horse will help veterinary ophthalmologists assess retinal function of clinical patients with congenital and acquired ocular abnormalities. Additionally, these values will hopefully assist in characterization of inherited photoreceptor dystrophies and degenerations.

This study reports the results of scotopic and photopic ERG parameters in sedated horses obtained using a mini-Ganzfeld and a contact lens recording electrode with a protocol that adheres to ISCEV standards. There was a wide range of variability observed within the individual ERG parameters. This can be explained by the natural variability from one horse to the next or potentially from the technique used. To avoid the latter possibility, the same HMsERG unit, equipment, operator, and protocol including baseline and impedance testing were used on each horse. None of the horses in this study developed any adverse side effects from the sedation, ERG protocol, or equipment used. The methods used proved to be simple and safe for staff and resulted in consistent data collection.

Efficacy of IV detomidine for harvesting of ERG responses was assessed as a secondary goal of this study. The first two ERG responses recorded, low and middle light intensity, obtained accurate and easily interpreted data from all horses. With increasing time and light intensity, a few horses displayed movement away from the light stimulus. Therefore, we were unable to report each of the ERG responses in all of the study patients. High light intensity responses were not reported in two of 30 horses (7%), and cone flicker responses were not reported in three horses (10%). The descriptive statistics was calculated based on all reported data.

General anesthesia is often recommended for the proper recording of ERGs, especially for longer protocols based on the standards of the ISCEV, to avoid recording artifacts from muscle activity and to allow for ideal globe position.[1] Sedation is less expensive and labor-intensive as well as safer than general anesthesia, making it preferable for most

equine owners and practitioners. This study is in agreement with previous studies that have reported IV detomidine at 0.015 mg/kg as a safe and effective sedation choice for the use of advanced full-field flash ERG in the horse. The half life of IV detomidine in the horse is ~30 min with a reported sedation time lasting 60–150 min.[27] The total time required from sedation to removal of the electrodes for this protocol is ~45 min. It is possible that the sedative effects were weaning toward the end of the protocol when high light intensity and cone flicker response testing occurred. The sedation protocol used was simple and easy to perform; however, the disadvantage is that 7–10% of the horses in this study did not allow for complete testing owing to movement. The redosing or use of a constant rate infusion of detomidine is likely required for 100% compliance. Evaluation of multiple doses of detomidine hydrochloride was beyond the scope of this study.

Oscillatory potentials are small wavelets on the rising phase of the b-wave using high-intensity light stimulus.[1,4] They are derived from the inner nuclear layer with the bipolar, amacrine, and interplexiform cells suspected to be directly or indirectly involved in their generation.[9,28] ISCEV recommends using an overall bandpass of 75–100 Hz on the low end and 300 Hz or above at the high end to record OPs.[4] Using these parameters, our study was unable to elicit distinct OPs. Only one study has reported the presence of OPs in the equine ERG. This study used a bandwidth of 100–500 Hz to filter out low-frequency waveforms.[9]

The standardization of ERG and sedation protocols is important for comparison studies and necessary for the extrapolation and interpretation of final results. Consistency should begin with obtaining baseline ERGs on normal horses prior to testing abnormal populations. Ideally, breed, gender, and age-matched studies should be performed as a- and b-wave AMP, and IT may differ.[2,8] This study reports results of the measured ERG parameters in normal thoroughbred mares using 0.015 mg/kg IV detomidine hydrochloride, the HMsERG, and a contact lens electrode. Potential future directions may include comparison studies using different sedation protocols or titrating doses and routes of detomidine hydrochloride as well as assessing different genders, breeds, age-matched, and abnormal populations of horses affected with congenital, heritable, immune-mediated, or acquired ophthalmic disease.

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