

Electroretinography in the western gray kangaroo (*Macropus fuliginosus*)

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Abstract

Objective To perform electroretinography on normal anesthetized western gray kangaroos (*Macropus fuliginosus*).

Animals studied Six captive western gray kangaroos.

Procedures The kangaroos were anesthetized using a combination of ketamine and medetomidine via a remote drug delivery system, then were maintained on isoflurane after endotracheal intubation and reversal of the medetomidine with atipamazole. After a minimum of 20 min of dark adaptation, electroretinograms were obtained using a handheld electroretinography (ERG) machine using a single flash protocol at three light intensities: 10 mcd.s/m², 3000 mcd.s/m², 10 000 mcd.s/m².

Results At 10 mcd.s/m² the mean b-wave amplitude and implicit time was 102.0 μ V (SD \pm 41.3 and 95% CI 68.9–135.1) and 78.4 ms (SD \pm 8.3 and 95% CI 71.8–85.0). At 3000 mcd.s/m² the mean a-wave amplitude and implicit time was 69.9 μ V (SD \pm 20.5 and 95% CI 53.5–86.3) and 17.6 ms (SD \pm 1.5 and 95% CI 16.4–18.8) and the mean b-wave amplitude and implicit time was 175.4 μ V (SD \pm 35.9 and 95% CI 146.7–204.1) and 74.1 ms (SD \pm 3.5 and 95% CI 71.2–76.9). At 10 000 mcd.s/m² the mean a-wave amplitude and implicit time was 89.1 μ V (SD \pm 27.1 and 95% CI 67.5–110.8) and 16.8 ms (SD \pm 1.0 and 95% CI 16.0–17.0) and the mean b-wave amplitude and implicit time was 203.7 μ V (SD \pm 41.4 and 95% CI 170.6–236.8) and 75.4 ms (SD \pm 3.3 and 95% CI 72.8–78.1).

Conclusion Electroretinography outside of the typical clinical setting is feasible using a portable ERG system and allows for quick analysis of retinal function in exotic species.

Key Words: electroretinogram, eye, macropod, *Macropus fuliginosus*, retina, western gray kangaroo

BACKGROUND

Electroretinography (ERG) is commonly utilized in veterinary ophthalmology to assess and document retinal electrical function. The first reports of canine and feline ERGs occurred in the early and mid 20th century.^{1–3} ERG is used in research settings for the study of inherited and acquired retinopathies, in clinical settings for differentiating retinal vs. central nervous system disease in cases of acute vision loss and for evaluating retinal electrical function in patients with opaque ocular media that precludes direct examination of the posterior segment.^{4,5} The ERG represents a complex summation of electrical potentials and currents generated within the cells of the retina. The negative deflection of the

a-wave is produced by the hyperpolarization of photoreceptors as they undergo phototransduction, whereas the b-wave is produced primarily by bipolar cells.^{6–8} Traditional ERG units are bulky and nonportable, however within the last 10 years several portable ERG systems have become commercially available, making electrodiagnostic assessment of nontraditional patients possible outside of the traditional research laboratory or clinical setting.

Western gray kangaroos (*Macropus fuliginosus*) are macropods native to Southwestern Australia. With weights from 28 to 54 kg, heights up to 1.1 m, and tail lengths from 80 to 100 cm, they are one of the largest species of kangaroo. Males are generally twice as large as females, however they are all herbivorous. Although western gray kangaroos are

commonly housed in zoological collections, there is a paucity of information about the normal ophthalmic examination findings and common ocular diseases of this species. Published reports include a review of common marsupial ocular diseases, a single report of retinal degeneration in a Goodfellow's tree kangaroo (*Dendrolagus goodfellowii*) and several detailed descriptions of a viral outbreak of anterior uveitis, chorioretinitis, optic neuritis and encephalitis causing vision loss in Australian kangaroos.⁹⁻¹³ A review of normal ocular examination findings in a captive mob of western gray kangaroos is also available.¹⁴

A retrospective evaluation of necropsy data on kangaroos housed in a single zoological collection revealed a high prevalence of histologic lesions compatible with hypertension, including renal arteriolar smooth muscle hypertrophy, extracellular matrix accumulation within renal arterioles, renal vascular tortuosity, juxtaglomerular hyperplasia and hypertrophy of arterioles and arteries in both the retina and central nervous system.¹⁵ Hypertension is frequently associated with retinopathy, particularly in the cat.¹⁶ A recent report identified decreased b-wave amplitudes in spontaneously hypertensive rats.¹⁷ Establishing a protocol for ERGs in western gray kangaroos may be a useful part of evaluating a mob for hypertension and its associated ocular lesions. The goal of this study was to demonstrate a method for performing ERGs in an exotic species in a nonclinical setting.

MATERIALS AND METHODS

The study protocol was approved by the Brookfield Zoo Research and Scientific Committee and Institutional Animal Care and Use Committee. A mob of six captive adult western gray kangaroos (three males and three females) were included in the study, ranging in age from 3 to 7 years and in weight from 22.4 to 67.5 kg. All kangaroos were considered to be in good health at the time of the study with no evidence of visual deficits. Each kangaroo was anesthetized using a remote drug delivery system (Telinject; Telinject USA, Inc., Agua Dulce, CA, USA) with 2 mg/kg ketamine hydrochloride (Ketaset; Fort Dodge Animal Health, Fort Dodge, IA, USA) and 50 µg/kg medetomidine hydrochloride (Domitor; Pfizer Animal Health, Exton, PA, USA) administered intramuscularly with doses based on the most recently available body weight of the animal (information no older than 3 months for any kangaroo). The anesthetic drugs were administered within the kangaroo enclosure while the animals were free-ranging, and when the kangaroo became recumbent and immobilized, it was transferred to a building within the kangaroo enclosure. Each kangaroo was carefully monitored while under anesthesia, including heart rate and rhythm, direct and indirect blood pressure, arterial oxygen saturation and respiratory rate. After obtaining direct and indirect blood pressure measurements as part of an unrelated study, each kangaroo was administered 5% isoflurane (Isoflo; Abbott Animal Health, Abbott Park, IL, USA) by facemask with oxygen at 2-3 L/min. Each kangaroo was

then blindly intubated using a 6.0 or 7.0 mm internal diameter endotracheal tube, and isoflurane was continuously administered at concentration of 1-3% with 2-3 L/min of oxygen. Atipamazole (Antisedan; Pfizer Animal Health) was then administered intramuscularly at a dose of five times that of the previously administered medetomidine, and the kangaroo was maintained on isoflurane during the ERG. The amount of time between administration of the atipamazole and the start of the ERG varied from 10 to 16 min. Atipamazole is reported to reverse sedation and analgesia in dogs within 5-10 min, and these effects are extrapolated in western gray kangaroos as no pharmacologic studies are available in this species.¹⁸ After conclusion of the ERG and all additional anesthetic monitoring, administration of isoflurane and oxygen was discontinued and the kangaroos were moved to a dark and quiet recovery area where extubation was performed at the first sign of swallowing or purposeful movement. No regurgitation or vomiting was noted in any kangaroo.

Each kangaroo in this study had received a complete ophthalmic examination under general anesthesia 3 months prior to this study. Complete ophthalmic examination included diffuse illumination, slit lamp biomicroscopy (Kowa-SL2; Kowa, Tokyo, Japan), and indirect funduscopy (Keeler Instruments Inc., Broomall, PA, USA) with a 2.2D handheld condensing lens (PanRetinal 2.2; Volk Optical, Inc., Mentor, OH, USA). Intraocular pressure (IOP) was estimated using rebound (Tonovet; Icare Finland Oy, Espoo, Finland) and applanation tonometry (Tonopen-XL; Reichert Inc., Depew, NY, USA).¹⁴

No ophthalmic abnormalities were observed in any kangaroo included in the ERG study group that could potentially impact the ERG. Observed abnormalities did include a subepithelial scar, incipient anterior cortical cataract, eyelid notch defect, nuclear sclerosis and vitreal degeneration. One kangaroo in the mob was observed to have a regional focal choroidal hypoplasia and an optic nerve coloboma, however that kangaroo was not included in this study.¹⁴

Since all kangaroos had been examined 3 months prior to this study, complete ophthalmic examination was not performed prior to the harvesting of ERGs in this study to avoid the deleterious effects of excessive light exposure on the ERG.¹⁹ Tonometry with applanation and rebound tonometry was performed prior to the instillation of tropicamide 1% solution (Tropicamide 1% USP; Alcon Laboratories, Inc., Fort Worth, TX, USA) for mydriasis. Dark adaptation times varied from 20 to 60 min prior to beginning ERG. Since complete darkness was not possible for the entirety of the time of dark adaptation due to the need for continued anesthetic monitoring and the complex intubation process, dark adaptation was achieved by using adhesive tape to close the eyelids of each kangaroo and then taping a patch of dark material over the eyelids to simulate a dark environment. This technique allowed for the necessary pre-ERG dark adaptation without interfering with the work of the anesthetic team and monitoring. After dark adaptation was

concluded (minimum of 20 min), the ERG was performed using a monopolar electrode-contact lens (ERG-jet; Nicolet Instruments, Madison, WI, USA) applied to the cornea with hypromellose 2.5% gel as a coupling agent (Gonak; Akorn, Inc., Buffalo Grove, IL, USA), male subdermal platinum needle electrodes (FD-E2-24; Astro-Medical, Inc. Warwick, RI, USA) and a portable ERG machine, the Handheld Multispecies ERG (HM sERG) (HM sERG Model 1000; RetVetCorp, Columbia, MO, USA). The ground electrode needle was placed at the apex of the occiput directly between the two ears, and the reference needle was placed approximately 2 cm lateral to the lateral canthus of the eye being tested.

The Quick Ret Check protocol was used in both eyes of all kangaroos. The Quick Ret Check Protocol, which is part of the software of the HM sERG unit, was developed by Dr. Kristina Narfström. The HM sERG unit utilizes a white flash within a mini-Ganzfeld for stimulation of the retina. It utilizes only three levels of light stimuli in order to obtain an overall evaluation of retinal function very quickly with variable flash durations of 0.005–5 ms, depending on the test protocol. The first set consists of the average response to four light flashes (2 s in between flashes) at 10 mcd.s/m², followed by a single flash at 3000 mcd.s/m² and, after 20 s, another single flash at 10 000 mcd.s/m².²⁰ In dark adapted conditions ‘pure’ rod responses are obtained through the first set of low light intensity flashes, while for the second and third flash stimuli, the responses are derived from a mixture of rod and cone photoreceptors. The bandpass of the HM sERG unit was set at 0.3–300 Hz.

During the ERG, all ambient lights were turned off with the exception of anesthetic monitoring equipment monitors, which remained on but were positioned away from the kangaroo’s head, and a single 20 watt red light which was used to illuminate the kangaroo’s head and the ERG equipment. Ambient light intensity was not measured. The room in which all ERGs were performed was not insulated in any way from external electrical interference. Both cellular telephones and laptop computers were present within the examination room. At the conclusion of ERG, each kangaroo received a brief anterior segment examination using diffuse illumination and indirect funduscopy using a 2.2D condensing hand lens to ensure that no new ophthalmic lesions had developed since the previous examination 3 months prior to the harvesting of the ERGs.

All ERGs were analyzed using the HM sERG software, and a-wave and b-wave amplitudes and implicit times were recorded for each kangaroo. For the four kangaroos in which bilateral ERGs were obtained the data from the left and right eyes were combined and averaged for a single reading. The limited data set precluded inferential statistical testing, so mean, standard deviation (SD) and 95% confidence interval (CI) with alpha = 0.05 were reported for the amplitudes and implicit times of each ERG (Excel; Microsoft, Inc., Redmond, WA, USA).

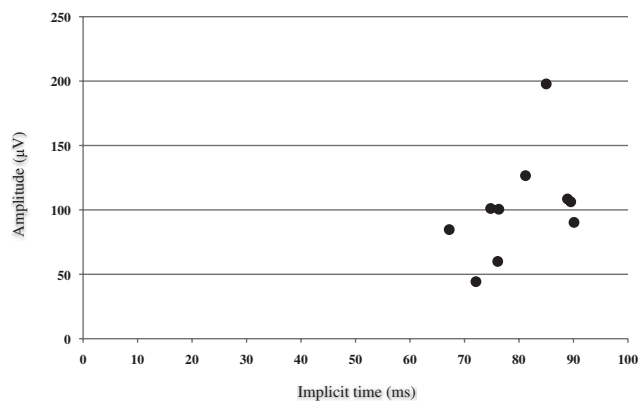


Figure 1. Scatter plot of the 10 mcd.s/m² ERG b-wave values. At this low light intensity, no a-wave values are recorded.

RESULTS

All kangaroos had normal ocular examinations at the time of ERG. Mean IOP as estimated with applanation tonometry was 12.9 mmHg with SD ± 10.5 mmHg and a range of 9–20 mmHg. Mean IOP as estimated with rebound tonometry was 10.1 with SD ± 4.8 mmHg and a range of 7–19.5 mmHg. Bilateral ERGs were obtained from all six kangaroos, however in two kangaroos (one male and one female), the reading in one eye was uninterpretable. All kangaroos were normotensive, normothermic and neither hypoxemic nor hypercapnic at the time of ERG harvesting. At 10 mcd.s/m² the mean b-wave amplitude and implicit times were 102.0 µV (SD ± 41.3 and 95% CI 68.9–135.1) and 78.4 ms (SD ± 8.3 and 95% CI 71.8–85.0) (Fig. 1). No a-wave is obtained in this low amplitude ERG response, which only consists of a low amplitude and late-onset b-wave, for which amplitude and implicit time data is reported. At 3000 mcd.s/m² the mean a-wave amplitudes and implicit times were 69.9 µV (SD ± 20.5 and 95% CI 53.5–86.3) and 17.6 ms (SD ± 1.5 and 95% CI 16.4–18.8) and the mean b-wave amplitude and implicit times were 175.4 µV (SD ± 35.9 and 95% CI 146.7–204.1) and 74.1 ms (SD ± 3.5 and 95% CI 71.2–76.9) (Fig. 2). At 10 000 mcd.s/m² the mean a-wave amplitude and implicit times and amplitudes were 89.1 µV (SD ± 27.1 and 95% CI 67.5–110.8) and 16.8 ms (SD ± 1.0 and 95% CI 16.0–17.0) and the mean b-wave amplitude and implicit times were 203.7 µV (SD ± 41.4 and 95% CI 170.6–236.8) and 75.4 ms (SD ± 3.3 and 95% CI 72.8–78.1) (Fig. 3). The normal ERG of kangaroo #6 is presented as Fig. 4.

DISCUSSION

This report demonstrates a practical method of performing ERG on an exotic species. ERG has not previously been reported in the western gray kangaroo, however one report details the ERG findings and photoreceptor spectral sensitivities of a related macropod, the tamar wallaby (*Macropus eugenii*).²¹ Veterinary ERG has a wide variety of

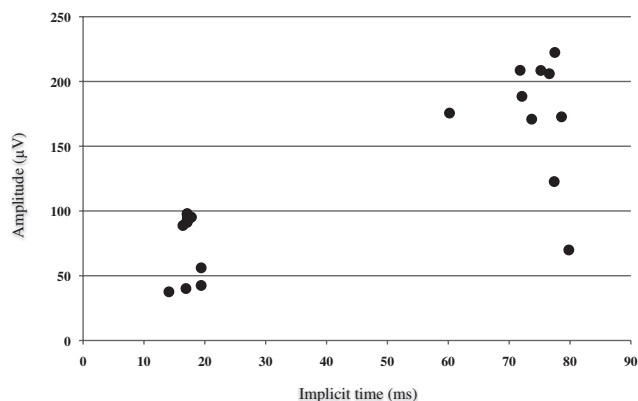


Figure 2. Scatter plot of the 3000 mcd.s/m² ERG values. The clustered values to the left represent a-wave values while the clustered values to the right represent b-wave values.

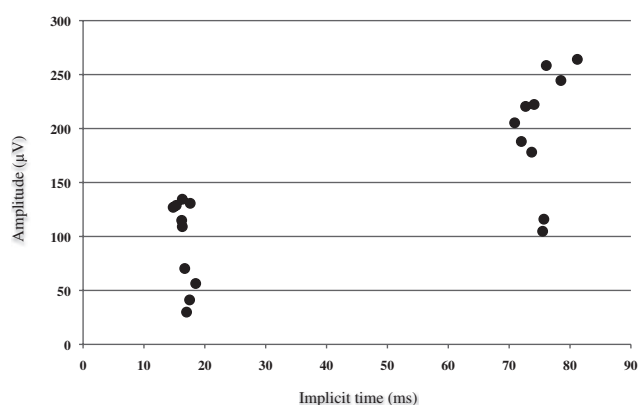


Figure 3. Scatter plot of the 10 000 mcd.s/m² ERG values. The clustered values to the left represent a-wave values while the clustered values to the right represent b-wave values.

applications, from the pre-operative screening of patients with mature cataracts prior to cataract surgery to complex diagnostic protocols for detecting subtle changes in rod/cone function in hereditary retinal degenerations. Although various research and clinical screening protocols have been well described, there is a paucity of information regarding ERG in exotic species. The size and bulk of most ERG machines have historically limited their field applications and use in exotic species that could not be examined in a hospital setting. With the development of a hand-held ERG machine such as the HM_sERG, the feasibility of performing ERG in exotic species housed in zoological collections has increased.

As phacoemulsification in exotic species has become more common, so does the need for accurate and accessible ERG.^{22–28} The purpose of this study was to describe a successful technique for field ERG in western gray kangaroos, however the technique has applications to a wide variety of exotic species, particularly those for which capture and transportation to a hospital for pre-operative evaluation is either not feasible or not in the best interest of the animal.

Kangaroos as a species are reported to be affected with nutritional cataracts, so establishing reference values of normal ERGs may be of significant diagnostic value.¹³

Electroretinography is plagued by a myriad of factors that interfere with accurate and reliable recordings of good quality. Such factors include excessive ambient electrical noise, inappropriate placement of ground and reference electrodes, faulty electrodes, poor contact between the corneal surface and the active electrode, irrelevant physiologic activity and inappropriate or faulty filters and amplifiers.²⁹ In this study, ERGs were unable to be obtained from one eye of two kangaroos. Although wide variety of physiologic, pathologic and pharmacologic factors also influence the ERG, including body temperature, oxygenation/ventilation status, IOP, and sedatives/anesthetics, these parameters were carefully monitored and are considered unlikely to have contributed to the poor ERG recordings.³⁰ Faulty electrodes and wiring are possible contributors to the poor ERG recordings, however the electrodes and wiring were successfully utilized for subsequent recordings, thus making this cause less likely. Excessive external electrical interference was considered a possible cause of the uninterpretable waveforms, however the electrical interference within the room (from external power cords and other electrical equipment) remained constant during the recording period, making this less likely. A more likely possibility is poor positioning of the reference electrode, causing amplification of background physiologic noise or poor contact between the JET electrode and the cornea. A possible improvement of the technique presented here would be to reposition reference electrodes when poor recordings were obtained, liberal application of the coupling media between the electrode and the cornea and minimization of the amount of ambient electrical interference by turning off unnecessary electrical devices. Future studies may investigate alternative corneal electrodes, including a microfiber electrode, that may be more appropriate for the curvature and size of the kangaroo cornea.³¹

In both human and veterinary ophthalmology, there has been a call for standards of uniformity in electrodiagnostic testing of retinal function, an attempt to make results from different laboratories or clinics more comparable.^{20,32} The protocol utilized in the study is modeled after the guidelines suggested in these consensus statements, however it differs from longer and more elaborate protocols in that it is meant to be a fast screening test and does not give detailed and complete information about rod and cone function separately. Cone function tests were not performed in this study because of time limitations with other phases of the study. The major advantage to the QuickRetCheck protocol used in this study is its brevity, which is ideal for use under field research or clinical conditions. The protocol has previously been utilized in dogs but has not been described in other species.³³ Other possible protocols that could be utilized that may have the same advantage of brevity include the shorter gross retinal function protocol proposed for use in the dog.³²

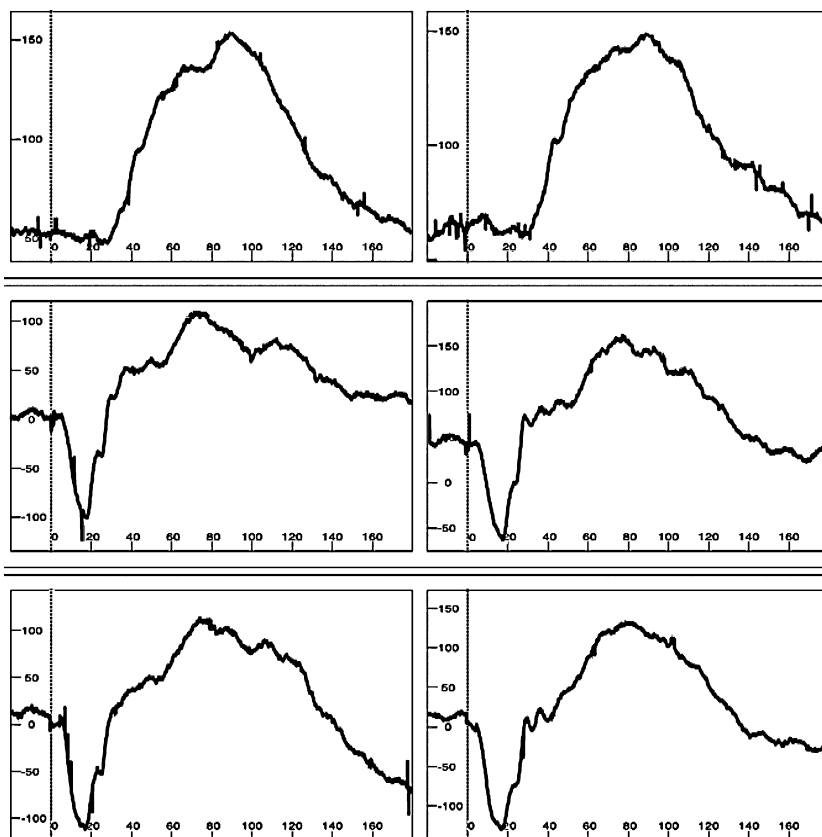


Figure 4. Representative recordings using the QuickRetCheck protocol in a 7-year-old western gray kangaroo. Bilateral recordings are shown with tracings from OS on the left side and tracings from OD on the right. For evaluation of the rod system (in the dark adapted state) the average of four flashes of low intensity light stimulation (10 cd.s/m^2) is used, followed by two single flashes of high intensity light stimulation, one flash at 3 cd.s/m^2 and another at 10 cd.s/m^2 . The latter two light stimuli show responses of mixed rod and cone photoreceptors.

The role of general anesthesia and its affect on the ERG is important for the accurate interpretation of the ERG. Medetomidine is reported to significantly but minimally prolong the implicit times and decreases the amplitudes of the canine ERG as evaluated using the HM_sERG.³³ The inhalant anesthetic gas isoflurane has been demonstrated to decrease the amplitudes of the a and b-waves compared to sedation with tiletamine-zolazepam in normal dark-adapted dogs, as have the inhalant gasses sevoflurane and halothane.^{34,35} The combination of thiopental and isoflurane has been shown to decrease the implicit time and amplitude of the a wave in normal dogs compared to the combinations of medetomidine and ketamine or xylazine and ketamine.³⁶ The anesthesia protocol in this case was selected as part of a related study on the affects of two anesthetic protocols on the blood pressure of western gray kangaroos. Medetomidine, an alpha-2-agonist and ketamine, a centrally acting N-methyl-D-aspartic acid receptor-inhibitor, are a commonly used combination for immobilization of western gray kangaroos. Since medetomidine is reported to cause peripheral hypertension in dogs, atipamazole was administered to the kangaroos in this study after beginning insufflation with isoflurane to eliminate this potentially confounding affect on measurement of blood pressure. The average time from administration of the ketamine/medetomidine to the beginning of the ERG was 64.2 min with a range of 40–83 min. The pharmacokinetics of the anesthetic agents used in this study have not been well

described in the western gray kangaroo, however in the dog, the average duration of action for medetomidine in the dog is approximately 30 min, and 20 min for ketamine.³⁷ It is less likely that either ketamine, given its duration of action, or medetomidine, given its duration of action and the administration of atipamazole as a reversal agent, significantly impacted the ERG, and therefore isoflurane is likely the largest influence on the ERG outcome in this group of anesthetized kangaroos. The implicit times of the b-wave at all light intensities were markedly increased compared to the identical ERG protocol with the HM_sERG machine in dogs sedated with only medetomidine, which may represent a drug effect or normal physiology for this exotic species.³³ It would be extremely difficult to perform an ERG in a nonanesthetized adult kangaroo due to their size and temperament, therefore the results of this study in anesthetized kangaroos provide a reasonable reference range for ERG values at three light intensities using a field ERG protocol. This study provides evidence that ERGs can be successfully performed in the zoological species using a portable ERG unit and an ERG protocol adapted for field purposes, opening up new avenues for future research in retinal electrophysiology.

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