

# Scotopic electroretinography in fishing cat (*Prionailurus viverrinus*) and leopard cat (*Prionailurus bengalensis*)

Metita Sussadee\*,†, Narathip Vorawattanatham,‡, Anuchai Pinyopummin,§, Janjira Phavaphutanon¶ and Aree Thayananuphat¶

\*Ophthalmology Unit, Kasetsart University Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand; †Department of Veterinary Technology, Faculty of Veterinary Technology, Kasetsart University, Bangkok 10900, Thailand; ‡Veterinary, Conservation and Research Section, Animal Management Division, Chiang Mai Night Safari, Chiang Mai 50230, Thailand; §Department of Large Animal and Wildlife Clinical Science, Faculty of Veterinary Medicine, Kasetsart University, Nakhon Pathom 73140, Thailand; and ¶Department of Companion Animal Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Nakhon Pathom 73140, Thailand

Address communications to:

A. Thayananuphat

Tel.: 66-867895505

Fax: 66-34351405

e-mail: fvetatn@ku.ac.th

## Abstract

**Objective** To establish baseline normal scotopic electroretinographic (ERG) parameters for two wild cat species: fishing cats (FC) and leopard cats (LC).

**Animal studied** Twelve normal, FC and eight LC kept in the Chiang Mai Night Safari Zoo, Thailand. The mean ages of FC and LC were 7.08 and 5.00 years, respectively.

**Procedure** All animals were studied using a standard scotopic protocol of a portable, handheld, multi-species electroretinography (HM sERG).

**Results** There were significant differences in the means of ERG b-wave amplitude of the rod response (Rod, 0.01 cd.s/m<sup>2</sup>), a- and b-wave amplitudes of standard light intensity of rod and cone response (Std R&C, 3 cd.s/m<sup>2</sup>) and b-wave amplitude of high light intensity of rod and cone response (Hi-int R&C, 10 cd.s/m<sup>2</sup>) with LC having higher amplitudes than FC. There was no significant difference in a- and b-wave implicit time except for the b-wave of Hi-int (P=0.03). No significant differences were observed in b/a amplitude ratios.

**Conclusions** Data from this report provides reference values for scotopic ERG measurements in these two wild cat species. It showed that the normal scotopic ERG responses have some differences between the two species which might be due to the skull conformation, eye size or physiology of the retina.

**Key Words:** electroretinography, fishing cat, leopard cat, retina, wild cat

## INTRODUCTION

The fishing cat (FC; *Prionailurus viverrinus*) and the leopard cat (LC; *Prionailurus bengalensis*) are small wild cats which reside in the forests of South-East Asia, including Thailand. Both wild cats are thought to play a role in the ancestry of domestic cats.<sup>1</sup> FC is a gray or olive-brown cat with spots less than 25 mm in width on the flanks, and LC is a buff or yellow cat with many dark spots over 25 mm in width on the back and flanks.<sup>1</sup> LC is about the same size as the domestic cat but a bit taller and typically weighs about 3–4 kg. LC is primarily nocturnal but can be active during the day. FC is comparatively larger and stronger than LC; its size and weight vary considerably, although normally it weighs about 7 kg.<sup>2,3</sup> FC is a strong swimmer and dives for its prey. FC is active at all hours of the day. The eyes of cats are the largest among

carnivores, which in addition to facing well forward, giving cats excellent stereoscopic vision.<sup>1</sup> Most cats including FC and LC are nocturnal and can see well at night. Hunting of these wild cat species is prohibited in many countries including Thailand as they are conserved animals and classified as endangered species.<sup>1</sup>

In domestic cats, electroretinography (ERG) can be used for the diagnosis of retinal diseases such as hereditary retinal degeneration,<sup>4–6</sup> noninflammatory retinopathy, and central retinal degeneration caused by dietary taurine deficiency.<sup>7</sup> Moreover, ERG has been used to investigate the retinal toxicity of antibacterial agents in cats.<sup>8,9</sup> Ophthalmologic studies in wild cat species are relatively limited. A few case reports in wild cats have revealed ocular defects and infection which were similar to those encountered in domestic cats.<sup>10,11</sup> ERG parameters in domestic cats using a short protocol have been reported.<sup>12</sup> However, there has

been no report for wild cat species; consequently, normal ERG values have not been published.

For this study, the two wild cat species, FC and LC, were investigated using animals of a similar range of ages. The examination environment, anesthetic protocol, and equipment were identical, leaving the species of animals as the only main variable. The aims of this study were to establish normal baseline ERG values and to evaluate any significant differences in ERG parameters between these two wild cat species.

## MATERIALS AND METHODS

Twelve FC and eight LC housed at the Chiang Mai Night Safari Zoo, Chiang Mai, Thailand, were investigated. Their ages ranged from 6–10 years (mean  $\pm$  SD =  $7.08 \pm 1.24$ ) for FC and 1–9 years (mean  $\pm$  SD =  $5.00 \pm 2.78$ ) for LC. The average weights of FC and LC were  $12.86 \pm 2.26$  kg and  $3.63 \pm 0.39$  kg, respectively. None of the animals used had a prior history of ocular abnormality or vision problems. The cats were given an intramuscular injection of 0.5 mg/kg xylazine (Ilium Xylazil-100, Ilium, Australia) to immobilize them in their enclosure and transferred to a darkened room for ERG recording. Pupils were dilated with tropicamide (1% Mydriacyl, Alcon, Belgium). They were induced with intravenous tiletamine–zolazepam (1.5 mg/kg, Zoletil, Virbac, France), and anesthesia maintained with isoflurane (1.5–2.5% Aerrane, Baxter Healthcare, Puerto Rico) delivered in oxygen (150 ml/kg/min).

Bilateral scotopic ERGs were recorded using a portable, handheld, multispecies electroretinograph (HM<sub>s</sub>ERG, OcuScience, Henderson, NV, USA) as previously reported.<sup>12</sup> Each eye was tested separately with the order randomized. A corneal electrode (ERG-jet, Fabrial SA, Switzerland) coupled with an artificial tear gel was used. A reference needle electrode was placed approximately 2 cm lateral to the lateral canthus, and a ground electrode placed over the occipital protuberance. The cats were dark adapted for 20 min and manipulations performed and anesthesia monitored under a dim red light. Three stimuli were used as follows: ‘Rod’ stimulus (0.01 cd.s/m<sup>2</sup>—four light flashes averaged); ‘Std R&C’ (3 cd.s/m<sup>2</sup>—single flash of standard light intensity to record mixed rod and cone responses); and ‘Hi-int R&C’ (10 cd.s/m<sup>2</sup>—single flash of high light intensity to record mixed rod and cone responses). The interstimulus interval time for Rod and Std R&C was 2 s, and between Std R&C and Hi-int R&C it was 10 seconds. After ERG recording, standard ophthalmic examination was performed using slit-lamp biomicroscopy, indirect ophthalmoscopy, and applanation tonometry. The median and mean ages, body weights, percentage isoflurane used, ERG amplitudes, ERG implicit times, and ERG b/a amplitude ratios were analyzed using SPSS 21 (SPSS Inc., Chicago, IL, USA) (IBM SPSS Statistics 21.0) (significance set a  $P < 0.05$ ). A *t*-test was used normally distributed data and a Mann–Whitney *U*-test for nonparametric data.

## RESULTS

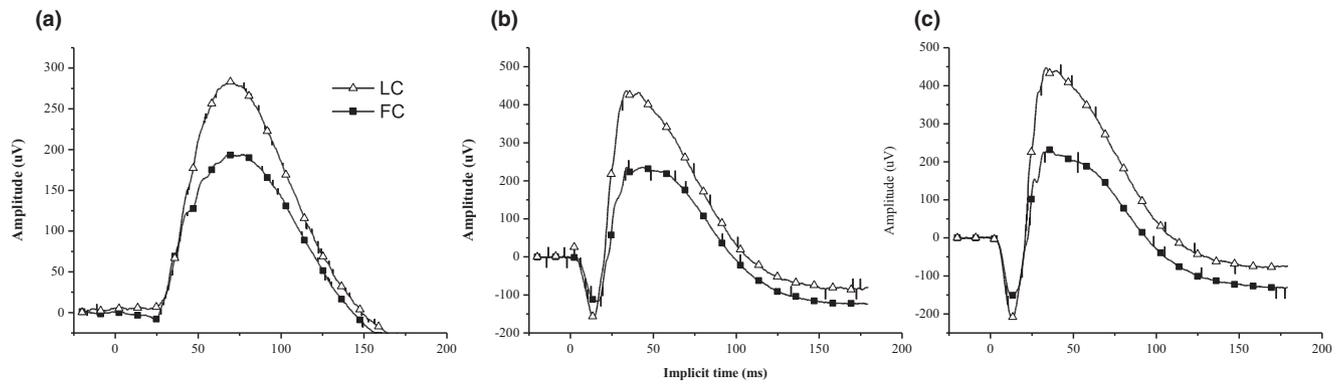
There was no statistically significant difference in the average age ( $P = 0.82$ ) and the percentage of isoflurane used ( $P = 0.73$ ). However, the average weight of the two species was significantly different ( $P < 0.01$ ). A small corneal scar in one eye of one LC was the only ocular abnormality observed post-ERG recording. The mean  $\pm$  SD intraocular pressures (IOP) of anesthetized FC and LC were  $14.16 \pm 2.76$  mmHg and  $18.75 \pm 2.62$  mmHg, respectively, which was significantly different ( $P < 0.01$ ). The fundus of all cats appeared normal on ophthalmoscopic examination.

ERGs were recorded from both eyes of all cats. There was no significant difference in the ERG measurements between the first and second eye. Typical ERG waveforms obtained from 5-year-old FC and LC are displayed in Fig. 1. A small a-wave was present in some ERG waveforms in response to the Rod stimulus in both FC and LC.

Normal ERG parameters for FC and LC are reported in Tables 1 and 2, respectively. There were significant differences in the means of ERG amplitudes between the two species (Fig. 2, a). LC had higher mean ERG amplitudes than FC for Rod b-wave ( $P < 0.01$ ), Std R&C a- and b-wave ( $P = 0.02$  and  $P < 0.01$ , respectively), and Hi-int R&C b-wave ( $P < 0.01$ ). However, there was no significant difference in the Hi-int R&C a-wave amplitude ( $P = 0.07$ ). The mean implicit times were not significantly different between the two species except for the Hi-int R&C b-wave implicit time which was significantly longer in the LC ( $P = 0.03$ ) (Tables 1 and 2, Fig. 2, b). The b/a amplitude ratio of Std R&C ( $P = 0.07$ ) and Hi-int R&C ( $P = 0.73$ ) was not significantly different between the two species.

## DISCUSSION

A guideline for clinical ERG in dogs recommended that each laboratory should obtain its own normal baseline ERG parameters.<sup>13</sup> Variations between ERGs can be due to the anesthetic technique,<sup>14–16</sup> electrode type and position,<sup>17</sup> and the age, species, and breed of the animal.<sup>13,18</sup> A study comparing ERG in four dog breeds—Poodle, Labrador Retriever, Thai Ridgeback, and Thai Bangkaew—reported that the breed of dog was an important variable in ERG, possibly based on the variation in skull conformation.<sup>19</sup> In feline species, a previous report on the evaluation of skull and mandible shape indicated that the skull shape was size-dependent.<sup>20</sup> There was a significant difference of the average body weights for each species in this study. Therefore, the variation in skull morphology and size might influence the skin reference electrode positioning. In addition, the eye size may be proportional to the body size, and hence, the axial length of FC eyes is greater than that of LC.<sup>21</sup> The retina in different eye sizes



**Figure 1.** Comparison of typical ERG waveforms of normal, 5-year-old, fishing cat and leopard cat: rod response to a 0.01 cd.s/m<sup>2</sup> flash (Rod) (a), mixture of rod and cone photoreceptors response to a 3 cd.s/m<sup>2</sup> flash (Std R&C) (b), and mixture of rod and cone photoreceptors response to a 10 cd.s/m<sup>2</sup> flash (Hi-int R&C) (c). Note the small a-wave of rod response in FC (a) and many small spike artifacts likely generated by the recording equipment in FC and LC (a, b, c).

**Table 1.** Normal ERG parameters in fishing cats; data presented as the median of ERG amplitude, implicit time, b/a ratio and range of the 5th and 95th percentiles

Light intensity	a-Wave amplitude (µV)	a-Wave implicit time (ms)	b-Wave amplitude (µV)	b-Wave implicit time (ms)	b/a ratio
'Rod' 0.01 cd.s/m <sup>2</sup>	None	None	103.20 (71.46–197.93)	70.95 (63.69–79.38)	None
'Std R&C' 3 cd.s/m <sup>2</sup>	88.05 (49.74–149.64)	16.00 (14.12–17.10)	358.05 (311.35–426.35)	41.00 (32.70–55.21)	4.10 (2.69–7.98)
'Hi-int R&C' 10 cd.s/m <sup>2</sup>	111.35 (75.89–192.49)	13.45 (11.70–16.66)	359.20 (307.91–423.17)	35.50 (31.92–42.41)	2.82 (2.15–4.55)

**Table 2.** Normal ERG parameter in leopard cats; data presented as the median (µV) of ERG amplitude, implicit time, b/a ratio and range of the 5th and 95th percentiles

Light intensity	a-Wave amplitude (µV)	a-Wave implicit time (ms)	b-Wave amplitude (µV)	b-Wave implicit time (ms)	b/a ratio
'Rod' 0.01 cd.s/m <sup>2</sup>	None	None	238.2 (125.35–382.78)	72.75 (63.23–77.90)	None
'Std R&C' 3 cd.s/m <sup>2</sup>	119.00 (102.70–210.80)	15.00 (12.98–16.88)	604.40 (455.13–766.68)	43.85 (35.33–53.15)	4.70 (3.11–6.63)
'Hi-int R&C' 10 cd.s/m <sup>2</sup>	176.65 (140.30–289.58)	14.10 (11.45–15.32)	649.3 (520.88–829.30)	39.70 (34.25–53.93)	3.54 (2.45–4.66)

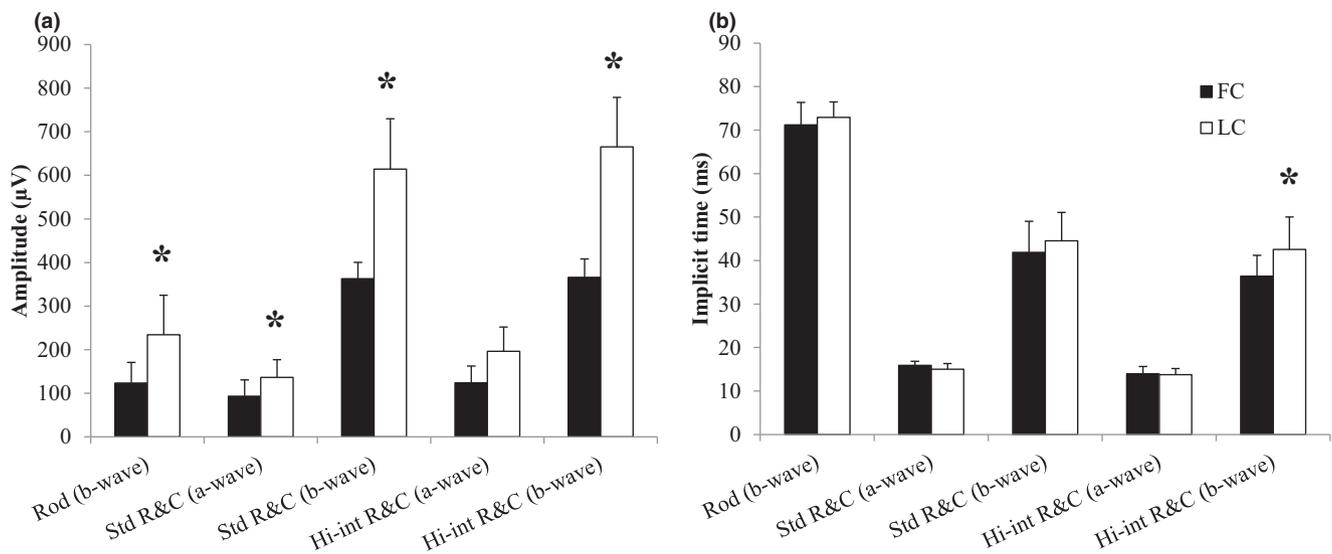
might be unequally illuminated by the HMsERG-ganzfeld. These factors might contribute to differences in the ERG baseline values between the two wild cat species.

The b/a amplitude ratio has been established in dogs<sup>22,23</sup> and cats.<sup>6</sup> It reflects signal transmission at the level of the photoreceptors and ON-bipolar cells.<sup>24</sup> In this study, there was no significant difference in the b/a ratio between the two species which implies that there might be no difference in the retinal signal transmission at the level of photoreceptors to ON-bipolar cells between these two species. The b/a ratio has also been investigated as a parameter for the early detection of retinopathy in cats.<sup>6</sup>

Normal ERG in domestic shorthair cats (DSH) was studied using an HMsERG in our previous report.<sup>12</sup>

When compared to DSH, the ERG waveforms obtained in the current study were similar except for the appearance of a small a-wave amplitude in some of the Rod responses in both FC and LC. The b-wave amplitude in response to the Rod stimulus of FC was similar in amplitude to that in DSH while in the LC it was markedly larger. The a-wave amplitudes of DSH were lower than in the FC and LC. Although the LC is a similar size to DSHs, the ERG amplitudes were higher. The a- and b-wave implicit times of the wild cats were similar to domestic cats.<sup>12</sup>

Adequate and appropriate general anesthesia was necessary to record ERG in wild cats to minimize artifacts from involuntary muscle activity and to ensure the safety of the researchers. Tiletamine-zolazepam and isoflurane have



**Figure 2.** Comparison of mean a- and b-wave amplitudes (a) and mean implicit times (b) from three flash levels of light stimulation in fishing cats and leopard cats. The error bars show  $\pm$ SD, and asterisks (\*) indicate significant differences between the cat species.

previously been used for ERG recordings from dogs and cats.<sup>15,25</sup> In this study, anesthesia induction with tiletamine–zolazepam followed by maintenance with isoflurane provided good immobilization. Although anesthesia can alter the ERG waveforms,<sup>15</sup> use of a standardized protocol allows for comparison of waveforms between different animal.

In conclusion, reference values of scotopic ERG for FC and LC using a portable ERG unit were established. ERG parameters of FC and LC were compared. The b-wave amplitudes of ERG between the 2 wild cat species were significantly different while almost all ERG implicit times were similar.

#### ACKNOWLEDGMENTS

This work was financially supported by The Kasetsart University Research and Development Institute (KURDI) for the project ‘Genetic Characteristics and Reproductive Physiology of Fishing Cat (*Prionailurus viverrinus*)’. The author would like to thank all the veterinarians and officers at the Chiang Mai Night Safari Zoo for their cooperation.

#### REFERENCES

- Lekagul B, Mcneely JA. *Mammals of Thailand*, 2nd edn. Bangkok, Thailand, 1988.
- Pollard M. *The Encyclopedia of Cats*, 2nd edn. Parragon Queen Street House, UK, 2002.
- Sunquist ME, Sunquist F. *Wild Cats of the World*. University of Chicago Press, Chicago, IL, 2002.
- Ekesten B, Narfström K. Abnormal dark-adapted ERG in cats heterozygous for a recessively inherited rod-cone degeneration. *Veterinary Ophthalmology* 2004; **7**: 63–67.
- Narfström K, Wile NM, Andersson BE. Hereditary retinal degeneration in the Abyssinian cat: developmental studies using clinical electroretinography. *Documenta Ophthalmologica* 1988; **69**: 111–118.
- Vaegan, Narfström K. Optimal discrimination of an Abyssinian cat recessive retinal degeneration: a short electroretinogram protocol is more efficient than a long one. *Clinical and Experimental Ophthalmology* 2004; **32**: 619–625.
- Jacobson SG, Kemp CM, Borruat FX *et al.* Rhodopsin topography and rod-mediated function in cats with the retinal degeneration of taurine deficiency. *Experimental Eye Research* 1987; **45**: 481–490.
- Ford MM, Dubielzig RR, Giuliano EA *et al.* Ocular and systemic manifestations after oral administration of a high dose of enrofloxacin in cats. *American Journal of Veterinary Research* 2007; **68**: 190–202.
- Messias A, Gekeler F, Wegener A *et al.* Retinal safety of a new fluoroquinolone, pradofloxacin, in cats: assessment with electroretinography. *Documenta Ophthalmologica* 2008; **116**: 177–191.
- Barnett KC, Lewis JC. Multiple ocular colobomas in the snow leopard (*Uncia uncia*). *Veterinary Ophthalmology* 2002; **5**: 197–199.
- Kik MJ, Van der Hage MH, Greydanus-van der Putten SW *et al.* Chlamydiosis in a fishing cat (*Felis viverrina*). *Journal of Zoo and Wildlife Medicine* 1997; **28**: 212–214.
- Sussadee M, Phavaphutanon J, Ubolrat K *et al.* Normal electroretinogram in domestic shorthair cats using a short protocol of HMsERG. *The Thai Journal of Veterinary Medicine* 2014; **44**: 237–242.
- Ekesten B, Komaromy AM, Ofri R *et al.* Guidelines of clinical electroretinography in the dog: 2012 update. *Documenta Ophthalmologica* 2013; **127**: 79–87.
- Jeong MB, Narfström K, Park SA *et al.* Comparison of the effects of three different combinations of general anesthetics on the electroretinogram of dogs. *Documenta Ophthalmologica* 2009; **119**: 79–88.
- Lin SL, Shiu WC, Liu PC *et al.* The effects of different anesthetic agents on short electroretinography protocol in dogs. *The Journal of Veterinary Medical Science* 2009; **71**: 763–768.

16. Norman JC, Narfstrom K, Barrett M. The effects of medetomidine hydrochloride on the electroretinogram of normal dogs. *Veterinary Ophthalmology* 2008; **11**: 299–305.
17. Mentzer AE, Eifler DM, Ferreira FM *et al.* Influence of recording electrode type and reference electrode position on the canine electroretinogram. *Documenta Ophthalmologica* 2005; **111**: 95–106.
18. Itoh Y, Maehara S, Itoh N *et al.* Electroretinography recordings using a light emitting diode active corneal electrode in healthy beagle dogs. *Journal of Veterinary Science* 2013; **14**: 77–84.
19. Sussadee M, Phavaphutanon J, Kornkaewrat K *et al.* Normal clinical electroretinography in poodle, labrador retriever, Thai ridgeback and Thai bangkew dogs. *Journal of Veterinary Science* 2015; **16**: 67–74.
20. Christiansen P. Evaluation of skull and mandible shape in cats (Carnivora: Felidae). *PLoS One* 2008; **3**: 1–8.
21. Ross CF, Kirk EC. Evolution of eye size and shape in primates. *Journal of Human Evolution* 2007; **52**: 294–313.
22. Lee J, Kim K, Jang H *et al.* The normal electroretinogram in adult healthy Shih Tzu dogs using the HMSeERG. *Journal of Veterinary Science* 2009; **10**: 233–238.
23. Maehara S, Itoh N, Itoh Y *et al.* Electroretinography using contact lens electrode with built-in light source in dogs. *The Journal of Veterinary Medical Science* 2005; **67**: 509–514.
24. Fitzgerald KM, Cibis GW, Giambrone SA *et al.* Retinal signal transmission in Duchenne muscular dystrophy: evidence for dysfunction in the photoreceptor/depolarizing bipolar cell pathway. *The Journal of Clinical Investigation* 1994; **93**: 2425–2430.
25. Hellyer P, Muir WW, Hubbell JA *et al.* Cardiorespiratory effects of the intravenous administration of tiletamine-zolazepam to cats. *Veterinary Surgery* 1988; **17**: 105–110.