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Normal Electroretinogram in Domestic Shorthair Cats Using a Short Protocol of HMsERG

Metita Sussadee^{1,2} Janjira Phavaphutanon³ Kanthapan Ubolrat^{1,2} Aree Thayananuphat^{3*}

Abstract

This study aimed to determine normal electroretinogram (ERG) parameters in healthy domestic shorthair (DSH) cats. Normal ERG in seventeen DSH cats was recorded using the QuickRetCheck protocol, a very short method to assess retinal function, of the Handheld Multispecies electroretinography (HMsERG). Scotopic ERGs were recorded in cats under tiletamine-zolazepam and isoflurane anesthesia. At low light intensity (10 mcd.s/m²) stimulation which determined the rod photoreceptor function, a-wave could not be detectable while the mean±standard error of b-wave amplitude and implicit time were 96.26 ± 5.98 μV and 64.66 ± 0.83 msec, respectively. At standard light intensity (3,000 mcd.s/m²), the mean a-wave amplitude and implicit time were 67.10 ± 4.08 μV and 16.20 ± 0.29 msec and the mean b-wave amplitude and implicit time were 413.38 ± 12.89 μV and 41.21 ± 0.95 msec, respectively. At high light intensity (10,000 mcd.s/m²) the mean a-wave amplitude and implicit time were 92.04 ± 5.03 μV and 15.49 ± 0.29 msec and the mean b-wave amplitude and implicit time were 444.34 ± 13.73 μV and 35.91 ± 0.94 msec, respectively. The b/a ratios of standard and high light intensity stimulation were 6.76 ± 0.39 and 5.09 ± 0.22, respectively. The present study successfully established normal ERG parameters in DSH cats.

Keywords: domestic shorthair cat, electroretinogram, general anesthesia, short protocol

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Introduction

Electroretinography (ERG) is a technique that objectively assesses the function of the retina. ERG procedures are broadly generated into two types, a very brief or short ERG protocol and a more comprehensive or long protocol (Ekesten, 2007). The short protocol is used to determine presence or absence of retinal response. This protocol is mainly for routine preoperative evaluation before cataract surgery. For the long protocol, identifying the rod and cone response can be used for early diagnosis of inherited photoreceptor atrophies (Maggs et al., 2008; Ekesten et al., 2013). Several studies on the usefulness of both ERG protocols for retinal diagnosis in cats affected with inherited retinal degeneration were reported (Narfström et al., 1988; Ekesten and Narfström, 2004; Vaegan and Narfström, 2004; Rosolen et al., 2005; Vaegan and Narfström, 2008). However, these studies reported only some parameters which were specific to the objectives of their studies including specific to ERG systems and breed of cats.

A few guidelines for clinical ERG techniques in animals were published. An ERG guideline for dogs was modified from the ERG protocol in human which was approved by the International Society of Clinical Electrophysiology of Vision (ISCEV) (Narfström et al., 2002; Marmor et al., 2009; Ekesten et al., 2013). An ERG guideline for rabbits was published in 2004 (Gjörloff et al., 2004). Until now, no official ERG guideline for cats has been published. In addition, complete ERG parameters of short ERG protocol in cats have never been studied. Numerous factors can distort ERG recordings such as stages of retinal adaptation, electrode types and position, anesthetic techniques, ages, species and breeds of the animal (Marmor et al., 2009). Therefore, the reported parameters could not be used as references for domestic short hair (DSH) cats. This study aimed to establish normal ERG parameters of adult healthy DSH cats using the short protocol under tiletamine-zolazepam and isoflurane anesthesia.

Materials and methods

Animal selection: Seventeen DSH cats were participated in this study. Ages ranged from 2 to 5 years (mean \pm SD = 3.23 \pm 1.03). All cats were housed and food was managed variously by their owners. Cats were only included if general physical and ocular examinations were normal with absence of previous ocular disease. Ocular examination was determined with standard equipments such as slit-lamp biomicroscopy, indirect ophthalmoscopy and applanation tonometer. All cats were assessed by a

very short ERG protocol under general anesthesia with the owners' consent.

Equipment and recording procedure: The subjects were fasted for at least 8 h and kept in a quiet dim light room for 2 h before performing ERG recording to reduce exposure to strong light which may affect the recording (Narfström et al., 2002). Pupils were fully dilated with tropicamide (Midriacyl, Alcon, Belgium) at 30 min intervals. Anesthesia was performed with 7 mg/kg tiletamine-zolazepam (Zoletil, Verbac, France) intramuscularly. General anesthesia was maintained with isoflurane (Aerrane, Baxter Healthcare, Puerto Rico) delivered with oxygen at dial setting for isoflurane vaporizer from 0.25 to 0.75% via an endotracheal tube. All cats were placed in sternal recumbency in a dark quiet room for 20 min for dark adaptation. Topical anesthetic eye drop (0.5% tetracaine hydrochloride ophthalmic solution, Alcon, Belgium) was applied. A lid speculum was applied to open the eyelids. Corneal electrode (ERG-jet, Fabrial SA, Switzerland) was positioned with artificial tear solution (Methocel 2%, OmniVision, Germany) applied between the corneal surface and the contact lens. The reference needle electrode was placed approximately 2 cm lateral to the lateral canthus of the eye. The ground needle electrode was placed over the external occipital protuberance directly between two ears. All electrodes were connected to a preamplifier and signals were amplified with a bandpass filter between 0.3 and 300 HZ of the mini-Ganzfeld Handheld Multi-species ElectroRetinoGraph (HM sERG, Xenotec, USA). Electrode Placement and anesthetic monitoring were performed under dim red light. The mini-Ganzfeld was positioned as close to the eye as possible without touching the cat to minimize the effect of scattered light. Before ERGs were recorded, the baseline and impedance were verified. The protocol used in this study was the QuickRetCheck protocol which was a part of the software of the HM sERG unit. Light stimuli were generated by white LEDs in Mini-Ganzfeld flash Dome of which its intensities were shown in Table 1. Low light intensity stimuli were generated to evaluate rod function. A standard light intensity stimulation was generated to evaluate both rod and cone function. A high intensity light stimulus was flashed to clarify a-wave when it was unclear with a standard light intensity stimulation. All steps were performed in scotopic test condition. The entire time for ERG protocol in one eye took 18 seconds. ERG parameters in both eyes of all cats were recorded.

Evaluation of ERG: Amplitude of the a-wave was measured in micro-volt (μ V) from the baseline to the

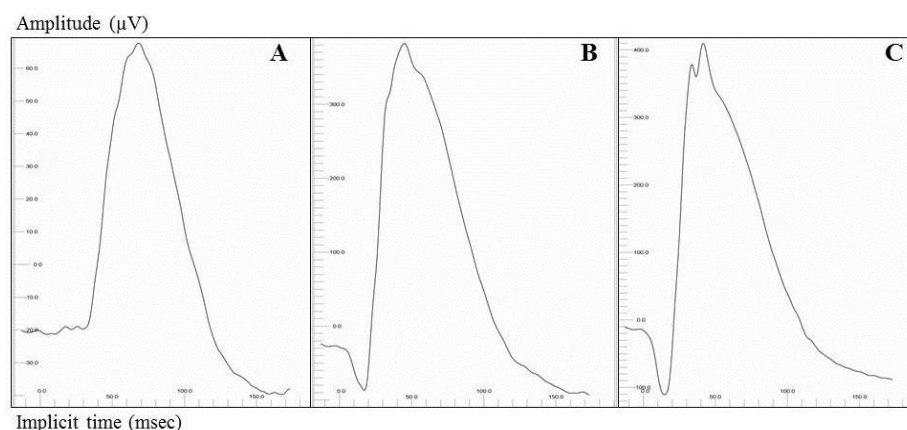
Table 1 ERG test sessions: intensity of light and time for each ERG session of the short (QuickRetCheck) protocol of HM sERG (Modified from a user manual for HM sERG instrument, 2006)

Session	ERG Test Sessions	Flash Intensity (mcd.s/m ²)	Number of Flashes	Time required (second)	Elapsed Time (second)
1	Low light intensity	10	4	8	8
2	Standard light intensity	3,000	1	0	8
3	High light intensity	10,000	1	10	18

Table 2 Normal a- and b- wave amplitude and implicit time of ERG recordings in DSH cats using the short (QuickRetCheck) protocol of the HMsERG equipment

Light intensity	Response				
	a-wave		b-wave		b/a amplitude ratio
	amplitude (μV)	implicit time (msec)	amplitude (μV)	implicit time (msec)	
Low	N/A	N/A	96.26 \pm 5.98	64.66 \pm 0.83	N/A
Standard	67.1 \pm 4.08	16.2 \pm 0.29	413.38 \pm 12.89	41.21 \pm 0.95	6.76 \pm 0.39
High	92.04 \pm 5.03	15.49 \pm 0.29	444.34 \pm 13.73	35.91 \pm 0.94	5.09 \pm 0.22

N/A; not applicable

**Figure 1** Dark-adapted (scotopic) ERGs from a 2-year-old normal mixed-breed cat showing responses to three different light intensities; A: Response to low light intensity stimulation (10 mcd.s/m²), only b-wave was obtained corresponding to activity from the rod photoreceptor, B and C: Response to standard and high light intensity stimulation (3,000 and 10,000 mcd.s/m², respectively), both a- and b-waves were obtained demonstrating activities of both rod and cone photoreceptors.

peak of the first negative deflection. Amplitude of the b-wave was measured from the peak of the a-wave to the largest positive- trough. Implicit times (msec) of both waves were measured from the onset of the flash stimulus to the peaks of the a- or b-wave. Data from both eyes of each cat were combined and averaged for a single reading. Normal values of ERG waveforms in each response were statistically defined as mean \pm standard error and range of minimum to maximum values. b/a ratio were also calculated.

Result

All cats had no ocular abnormality. Mean intraocular pressure was within normal limit (19.06 mmHg \pm 4.14 mmHg) with a range of 11-25 mmHg. Subconjunctival stay suture was not required during performing ERG.

Normal ERG waveforms of a 2-years-old cat are presented in Figure. 1. At low light intensity stimulation, a-wave was undetectable (Fig 1A). B-wave appeared in all light intensity stimulation. The ERG waveforms of standard and high intensity stimulations were similar (Fig 1B, 1C). No statistically significant difference of ERG parameters between the left and right eyes was found. Normal parameters of the amplitude and implicit time of ERG in DSH cats were reported in Table 2.

Discussion

The present study successfully established normal ERG parameters in DSH cats using the short protocol. The a-and b-wave amplitudes in these cats were lower than a previous report in offspring of Abyssinian cats at low light intensity stimulation (1, 2 and 4 cd.s/m²) (Vaegan and Narfström, 2004). The b/a ratio in the present study was higher than previous reports (Vaegan and Narfström, 2004; Rosolen et al., 2005). However, these parameters were difficult to compare because ERG systems, equipment, and anesthetic protocols, ages and breeds of cats affected ERG parameters (Marmor et al., 2009). An actual reason for breed-related ERG parameter has not been fully understood. In dogs it may involve voltage of the ERG signal that varies in different skull size and depth of intervening bone between breeds (Narfström et al., 2002). Therefore, several factors should be considered when ERG parameters are interpreted.

The short ERG protocol was chosen in this study because it was designed to evaluate retinal function in a very short time. The rod system and combined rod and cone photoreceptor were evaluated by different flash intensity and duration. All light intensities used in this study were a part of suggestion by ISCEV to use in humans and other mammals (Marmor et al., 2009). However, the number of flashes of each light stimulus was less than suggestion.

The short ERG protocol was not only used for evaluation before cataract surgery and diagnosis of blindness disorders in dogs and cats, but it was also an efficient tool to determine diagnostic scores for differentiation of disease status of recessive retinal degeneration in Abyssinian cats at an early age (Vaegan and Narfström, 2004). However, it should not be used to diagnose generalized photoreceptor disease, since it does not provide a comprehensive test of specific rod and cone function. Diagnosis of retinal degeneration should be based on the long protocol (Narfström et al., 2002).

To perform ERG in cats, sedation or general anesthesia was strongly recommended to prevent muscular movement during recording. A lot of studies have been published on the effects of general anesthetics on ERG in dogs but the data in cats were limited (Imai et al., 1990; Yanase and Ogawa, 1997; Kommonen et al., 2007; Jeong et al., 2009; Lin et al., 2009; Varela et al., 2010). Tiletamine is a safe dissociative agent in cats (Hellyer et al., 1988). Zolazepam is a benzodiazepine closely related to diazepam. It was used to help reduce the risk of seizure during recovery and promote skeletal muscle relaxation (Thomas and Lerche, 2011). To date, no study on the effect of this combination agent on ERG in cats has been published. However, it was reported as a desirable choice for the short ERG protocol in dogs (Lin et al., 2009). Isoflurane may affect the mechanism of potential action and change the ERG wave in dogs (Lin et al., 2009). Volatile anesthetics such as methoxyflurane, halothane and enflurane decreased the amplitudes of a-wave and the first oscillatory component in albino rabbits in dose-dependent manners (Tashiro et al., 1986). However, anesthetic combination that was used in this study resulted in desirable ERG waveforms with no side effect to all cats. Therefore this anesthetic combination is appropriate to use for short ERG protocol in cats. The depth of anesthesia must be carefully monitored for precise ERG parameters because deep anesthesia resulted in hypoxia and hypercapnea which would affect ERG parameters (Niemeyer et al., 1982; Derwent and Linsenmeier, 2000).

Subconjunctival stay suture was not required in this study because there was no effect of anesthesia that rotated the eyeball downward and duration of the anesthesia was stable long enough for ERG recording by the short protocol. A lid speculum that was used in the present study to stabilize the globe during ERG recording might influence electrical interferences. However, the speculum was recommended to create optimal light passage in pediatric patients (Marmor et al., 2009; Ekesten et al., 2013).

In conclusion, normal ERG parameters were successfully established in DSH cats. The ERG protocol, anesthetic agents, and equipment used in this study resulted in desirable ERG waves which were very helpful for providing accurate assessment of the remaining retinal function in DSH cats especially with severe cataract or late stage of retinal degeneration. These parameters will be very useful for helping the evaluation of retinal diseases in DSH cats under the same ERG protocol.

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บทคัดย่อ

ภาพคลื่นไฟฟ้าจอตาปกติในแมวบ้านขนสั้นที่บันทึกด้วยเกณฑ์วิธีแบบสั้นด้วยเครื่อง HM sERG

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การศึกษานี้มีวัตถุประสงค์เพื่อกำหนดค่าพารามิเตอร์ปกติของภาพคลื่นไฟฟ้าจอตาในแมวบ้านขนสั้นสุขภาพดี ภาพคลื่นไฟฟ้าจอตาปกติในแมวบ้านขนสั้น 17 ตัวถูกบันทึกด้วยเกณฑ์วิธี QuickRetCheck ของเครื่องบันทึกคลื่นไฟฟ้าจอตาชนิดมือถือ ซึ่งเป็นวิธีประเมินการทำงานของจอตาแบบรวดเร็ว ทำการบันทึกคลื่นจอตาในที่มืดในแมวที่ได้รับการวางยาสลบด้วยโทเลทามีน โคลาซีแพมและไอโซฟลูเรน จากการกระตุ้นด้วยความเข้มแสงต่ำ (10 mcd.s/m²) ซึ่งเป็นการวัดการทำงานของเซลล์รับแสงชนิดแท่ง พบว่าไม่ปรากฏ a-wave ขณะที่ค่าเฉลี่ย (mean ± standard error) ของ b-wave amplitude และ implicit time เท่ากับ 96.26 ± 5.98 μV และ 64.66 ± 0.83 msec ตามลำดับ เมื่อกระตุ้นด้วยความเข้มแสงมาตรฐาน (3,000 mcd.s/m²) พบว่าค่าเฉลี่ยของ a-wave amplitude และ implicit times เท่ากับ 67.10 ± 4.08 μV และ 16.20 ± 0.29 msec และค่าเฉลี่ยของ b-wave amplitude และ implicit time เท่ากับ 413.38 ± 12.89 μV และ 41.21 ± 0.95 msec ตามลำดับ เมื่อกระตุ้นด้วยความเข้มแสงสูง (10,000 mcd.s/m²) พบว่ามีค่าเฉลี่ยของ a-wave amplitude และ implicit times เท่ากับ 92.04 ± 5.03 μV และ 15.49 ± 0.29 msec และค่าเฉลี่ยของ b-wave amplitude และ implicit time เท่ากับ 444.34 ± 13.73 μV และ 35.91 ± 0.94 msec ตามลำดับ ส่วนค่าเฉลี่ยของอัตราส่วน b/a เมื่อกระตุ้นด้วยความเข้มแสงมาตรฐานและความเข้มแสงสูง เท่ากับ 6.76 ± 0.39 และ 5.09 ± 0.22 ตามลำดับ การศึกษานี้ประสบความสำเร็จในการกำหนดค่าพารามิเตอร์ของภาพคลื่นไฟฟ้าจอตาปกติในแมวบ้านขนสั้น

คำสำคัญ: แมวบ้านขนสั้น ภาพคลื่นไฟฟ้าจอตา การให้ยาสลบทั่วไป เกณฑ์วิธีแบบสั้น

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