NOTE *Surgery*

Effects of Stimulus Intensity for Electroretinogram in Conscious Miniature Schnauzers

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ABSTRACT. The aim of this study was to determine the most effective light intensity for flash electroretinogram (ERG) examination in conscious dogs using ERG equipment with a contact lens electrode with a built-in LED light source. ERG was performed on the bilateral eyes of ten clinically healthy Miniature Schnauzers at 6 different intensities (0.025, 0.079, 0.25, 0.79, 2.5 and 7.9 cd·s/m^2) after dark adaptation for 20 min. With the increase in stimulus intensity, the most significant increase in a and b-wave amplitudes were observed at 2.5 cd·s/m^2 ($p<0.05$). As the intensity of light was increased, the implicit times of both waves significantly decreased. Therefore, the most effective intensity of stimulus was 2.5 cd·s/m^2 in the conscious Miniature Schnauzers. This suggests that this procedure would be applicable for evaluation of retinal function in conscious dogs, especially in high-risk patients.

KEY WORDS: canine, electroretinogram, stimulus intensity.

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The ERG is the most widely reported procedure in veterinary medical literature. In dogs, the ERG has been useful for diagnosis or evaluation of retinal function in cataracts, retinal disorders and cortical blindness [4, 9]. In particular, evaluation by ERG of retinal function in the presence of a dense cataract when the fundus is not visible is a valuable clinical use [2]. The European College of Veterinary Ophthalmologist (ECVO), a body that governs the specialty in Europe, has completed guidelines for a standard flash ERG recording protocol in dogs. When mixed rod and cone function is to be tested, these guidelines recommend that the dog be dark-adapted for 20 min under anesthesia and that the intensity of flash stimulus be 2–3 cd•s/m^2 [5]. One major difference between human and dog patients when performing ERG recording is that anesthesia may be needed in dogs. However, almost all anesthetics apparently exert some influence on the waveforms of an ERG [10, 11]. There are few reports regarding how to record ERGs for conscious and non-stressed dogs, especially in high-risk patients [7]. This study was designed to determine the most effective light intensity in ERG for evaluation of retinal function in conscious Miniature Schnauzers using ERG equipment with a contact lens electrode with a built-in LED light source.

Ten clinically healthy Miniature Schnauzers were used in this study. The mean ± SD body weights and ages were 6.4 ± 1.1 kg and 3.9 ± 1.7 years old, respectively. Eight of the dogs were male and two were female. The ophthalmic examination included pupillary light reflexes, examination of anterior and posterior segment by slit-lamp biomicroscopy and fundus by indirect ophthalmoscopy after mydriasis with 1% tropicamide (Mydriacyl®, Alcon Laboratories Inc., Puurs, Belgium). None of the animals had any ocular diseases or abnormalities. A RETIcom® (ROLAND Instrument, Germany), ERG measuring instrument was used. White light stimulation was generated via a contact lens electrode with a built-in high luminance diode (LED-electrode; Kooijman/Damhof ERG lens®, Medical Workshop BV, The Netherlands). The reference and ground electrodes were platinum subdermal needle electrodes (Grass Instrument Division®, Astro-Med, Inc., U.S.A.). The electrodes were connected to a preamplifier, and the signals were amplified and passed through a bandfilter at 1–300 Hz before input into a special-purpose computer. The signals were averaged using this computer to improve the signal-to-noise ratio. Examinations were performed under dim red light in a dark room. The animals' pupils were dilated with 1% tropicamide. After producing mydriasis, the animals were dark adapted for 20 min. The ground electrode was positioned over the external occipital protuberance, and the reference electrode was positioned at approximately 2 cm caudal to the lateral canthus. The LED-electrode was positioned on the cornea after topical anesthesia with 0.5% proparacaine (Alcaine®, Alcon, Puurs, Belgium) and protection with 0.3% hydroxypropyl methylcellulose (Artear®, Unimed Pharm., Seoul, South Korea). The examinations were performed without any anesthetics with only restraint by an assistant; forced restraint was avoided to prevent inducing stress in the dogs. ERG was performed on bilateral eyes at six different intensities of stimulation in the order of weaker to stronger degree (0.025, 0.079, 0.25, 0.79, 2.5 and 7.9 cd•s/m^2). Each measurement was performed four times for each condition. The interval time between flashes was 15 seconds in order not to light adapt the rods, and there was 5 min between each level of the stimulus light [5]. The Wil-

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coxon signed-rank test was used for statistical analysis of the reproducibility of the ERG data. The statistical significance of differences was determined with $p<0.05$ as the minimum level of acceptable significance.

Representative waveforms of ERGs recorded for a 20 min dark-adapted conscious dog are shown in Fig. 1. With an increase in stimulus intensity, both a- and b-wave amplitudes increased, and the maximum response was observed at 7.9 cd·s/m^2. However, the most significant responses were observed at 2.5 cd·s/m^2 (Table 1). As the intensity of light increased, the implicit time of both a- and b-waves gradually decreased. The implicit time of the a-wave was significantly decreased at 0.79, 2.5 and 7.9 cd·s/m^2, respectively; however, significant decreases of the b-wave implicit time were observed at 0.025, 0.079 and 0.25 cd·s/m^2, respectively.

This study obtained reproducible results from conscious dogs using an intensity of 2.5 cd·s/m^2, which is recommended by the ECVO. The short protocol recommended by the ECVO is intended to rapidly determine gross retinal function in dogs that are about to undergo cataract surgery or, for instance, in which the diagnosis of retinal versus central blindness is to be evaluated [5]. This test reflects mixed rod and cone function and consists of the response to a single high-intensity flash of 2.0–3.0 cd·s/m^2 [5]. If stimulation averaging is needed, not more than one flash every 10 seconds has been recommended in order not to light adapt the rods [5]. In this study, the interval time between flashes was 15 seconds because the stimulus intensity used was up to 7.9 cd·s/m^2. Amplitude and implicit time of both waves are important parameters for clinical ERG recording. The threshold of the a-wave has been reported to be 100-fold higher than that of the b-wave under dark adaptation or low light adaptation [1, 3]. Thus, at lower intensities, an a-wave with a high threshold is barely recorded and only a b-wave with a low threshold is recorded. Both a- and b-wave amplitudes reached a maximum at 7.9 cd·s/m^2, but the most significant results were shown at 2.5 cd·s/m^2. There were more oscillatory potentials and some artifacts at 7.9 cd·s/m^2 than any other intensity on most ERG recordings. We believe that there were some artifacts due to blinking or head movement of the dogs because of the excessively bright light at 7.9 cd·s/m^2. Therefore, the most effective stimulus intensity in conscious Miniature Schnauzers was considered to be 2.5 cd·s/m^2. This intensity coincided with the standard flash for dogs (2–3 cd·s/m^2) [5]. The reason why Miniature Schnauzers were used in this study was that a high prevalence of progressive retinal atrophy has been shown for the Miniature Schnauzer, especially in South Korea [8]. One study

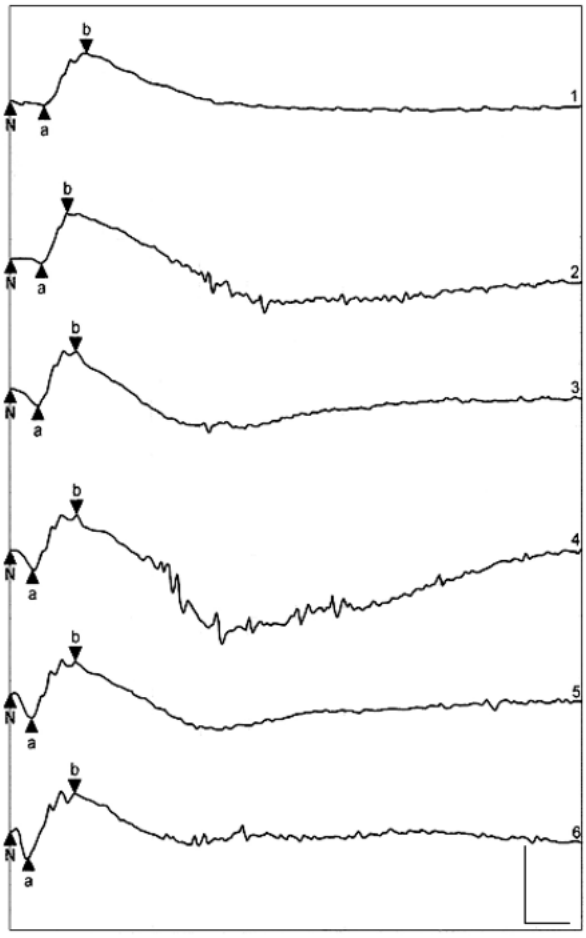

Fig. 1. Representative waveforms of ERG recording in conscious Miniature Schnauzers. N: onset of the stimulus. a: trough of a-wave. b: peak of b-wave. 1: stimulus intensity 0.025 cd·s/m^2. 2: 0.079 cd·s/m^2. 3: 0.25 cd·s/m^2. 4: 0.79 cd·s/m^2. 5: 2.5 cd·s/m^2. 6: 7.9 cd·s/m^2. Calibration; horizontal 25 ms, vertical 250 μV.

Table 1. Amplitude and implicit time of a- and b-waves at each stimulus intensity

Stimulus intensity (cd·s/m^2)	Amplitude (μV)		Implicit time (ms)	
	a wave	b wave	a wave	b wave
0.025	13.6 ± 12.8$^{a)}$	122.2 ± 44.4$^{a)}$	16.3 ± 4.2$^{a)}$	43.3 ± 4.7$^{a)}$
0.079	23.3 ± 20.6$^{b)}$	134.4 ± 35.6$^{a)}$	15.8 ± 2.4$^{a)}$	37.4 ± 5.8$^{b)}$
0.25	36.9 ± 18.9$^{c)}$	139.7 ± 40.6$^{ab)}$	14.7 ± 1.3$^{a)}$	33.4 ± 2.8$^{c)}$
0.79	66.9 ± 24.8$^{d)}$	150.9 ± 52.3$^{b)}$	13.3 ± 1.0$^{b)}$	31.8 ± 2.6$^{d)}$
2.5	87.8 ± 22.7$^{e)}$	189.7 ± 54.6$^{c)}$	11.3 ± 0.7$^{c)}$	31.7 ± 3.0$^{d)}$
7.9	96.5 ± 32.6$^{e)}$	192.1 ± 59.0$^{c)}$	10.3 ± 0.7$^{d)}$	33.4 ± 4.2$^{d)}$

a)-e): Different superscripts in the same column indicated statistical differences ($p<0.05$).
The data are expressed as Mean ± SD.

suggested that all animals should be anesthetized for a thorough ERG examination, and the effect for anesthetics on the recorded signal should also be noted [6]. Yanase and Ogawa [11] demonstrated that halothane anesthesia retards dark adaptation and reduces scotopic threshold response and b-wave amplitudes, but increases the amplitudes of the oscillatory potentials in the dog. Furthermore, the reaction might be variable according to the anesthetic agent, resulting in different effects on different retinal signals. For example, morphine may be used as a sedative in dogs, but in cats and horses it causes excitement, leading to potentially conflicting effects on ERGs [10]. A previous study reported that restraint of conscious dogs for recording of an ERG is very difficult, and the severe stress induced by forced restraint might lead to marked forms of damage, such as bodyweight reduction and hemorrhage in the stomach, in the dog [7]. In this study, however, no severe stress was deemed necessary to record ERGs for conscious animals.

This procedure would not be an adequate test of rod and cone function in patients that might be suffering from inherited photoreceptor disorders. But this protocol would be applicable for high-risk patients and for evaluation of retinal function before cataract surgery in conscious animals without sedation or anesthesia.

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