J Vet Clin 29(2) : 124-129 (2012)

Journal of Veterinary Clinics
www.ksvc.or.kr

Effect of Age and Gender on Electroretinogram in 34 Client-owned Healthy Dogs

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(Accepted: April 16, 2012)

Abstract : The purpose of this study was to identify the effect of age and gender on the value of electroretinogram (ERG) in healthy dogs. The ERG responses of 68 eyes of 34 dogs (22 males, 12 females) were recorded following the diagnostic protocol for dogs recommended by International Society for Clinical Electrophysiology of Vision under general anesthesia using medetomidine and tiletamine-zolazepam combination. There were significant differences in the implicit time of a-wave of Hi-int R & C response among age groups ($P < 0.05$) and in the implicit time of a-wave of cone response between male and female ($P < 0.05$). The rest ERG responses seem to be not affected by age and gender of healthy dogs.

***Key words* :** healthy dogs, ERG, age, gender.

Introduction

The full-field electroretinogram (ERG) is the record of electrical potentials that arise in retinal cells after light stimulation (4,13,21). ERG enables us to evaluate retinal function by providing information on which components of retinal cells are damaged through the analysis of electrical potential (3,4).

ERG can be applied in the following indications: 1) to evaluate retinal function of the candidates for cataract surgery to predict whether the patient's vision would be restored after surgery, whose lens opacity prevents an ophthalmoscopic evaluation of retina, 2) to diagnose various retinal disorders including inherited or acquired retinopathies such as sudden acquired retinal degeneration (SARD), progressive retinal atrophy (PRA), and rod and/or cone degeneration, in the early stage before ophthalmoscopic or clinical signs are presented, 3) to rule out the possibility of retinopathy and assure other neurologic problems including optic neuritis as the cause of blindness (4,13,21).

In order to secure consistency in the procedure of ERG measurement, International Society for Clinical Electrophysiology of Vision (ISCEV) established a standard for ERG in 1989, and lastly updated and revised these basic protocols in 2011 (14). The normal values established via these standardized process are essential for the analysis of ERG results and detection of retinal diseases. The standard of normal value in human was established according to the recommendation of ISCEV, and the effect of age and gender on ERG response was studied (2,5,7,12). In veterinary medicine, although there were reports on ERG values in healthy dogs (6,8,9,11,16,17, 19,22), the effects of diverse variables on ERG values including breed, age, gender, and other physical factors need to be further clarified.

The purpose of this study was to evaluate the effect of age and gender on ERG response in healthy dogs.

Materials and Methods

Animals

Sixty-eight eyes of 34 dogs (22 males, 12 females) whose owners volunteered for this clinical study and signed a consent form were evaluated. The questionnaires were supplied to the owners to find out history of dogs, especially ophthalmic disorder. The participating dogs included 2 Beagle, 2 Chihuahua, 10 Maltese, 2 Miniature Schnauzer, 7 Shih Tzu, 3 poodle, 2 Pekingese, 2 Yorkshire terrier, and 4 mixed dogs. Their average age was 6.3 ± 3.2 years. All dogs underwent physical examination, complete blood count and serum biochemical examination to rule out any systemic disease related to retinal lesion. Ocular abnormalities in all dogs were confirmed through ophthalmic examinations including pupillary light reflex, menace reflex, dazzle reflex, Schirmer's tear test, tonometry, slit lamp examination, direct ophthalmoscopy, indirect ophthalmoscopy, and ocular ultrasonography.

Anesthesia

The dogs were sedated with medetomidine hydrochloride (Domitor; Orion Pharma, Espoo, Finland, 40 μg/kg, SC). After 10 minutes, tiletamine-zolazepam (Zoletil 50; Virbac laboratories, Carros, France, 5 mg/kg, IV) was injected for general

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anesthesia. The dogs were positioned in sternal recumbence, and eyelid speculums were applied on their eyes. Half dose of tiletamine-zolazepam was administrated additionally when eye blinking or fine contraction of muscle was identified.

ERG measurement

1) ERG equipment- The portable mini-ganzfeld ERG unit (HMsERG; RetVetCorp, Columbia, MO, U.S.A.) that incorporates a stimulator and a recorder was used in this study. The reference and ground electrodes were needle electrodes (Model E2-straight needle; Astro-Med, Inc., WestWarwick, RI, U.S.A.) and a contact lens electrode (ERG-Jet; Nicolet Biomedical Instruments, Madison, WI, U.S.A.) was used as the active electrode. Obtained ERG recordings were stored on the compact flash card of the recording system, which were then transferred to a computer program (ERG viewer 2.1; RetVetCorp, Columbia, MO, U.S.A.) to be visualized and printed.
2) Animal preparation for ERG- The pupils were dilated with 0.5% tropicamide and 0.5% phenylephrine hydrochloride (Mydrin-P; Santen Pharmaceutical Co., LTD, Osaka, Japan) by two separate topical administrations at 15 minute interval to obtain a maximum mydriasis at least 1 hour before anesthesia. The ground electrode was placed on the external occipital protuberance and the reference electrodes were positioned at approximately 1 cm caudal to each lateral canthus. The active electrodes were positioned on cornea after administration of a topical anesthetic, 0.5% proparacaine hydrochloride (Alcaine; Alcon-Couvreur, Belgium). The artificial tear, 2% hypromellose (Hycel; Samil Pharm, Ansan, Gyenggi, Korea) were applied on active electrode of protection of cornea. The mini-ganzfeld flash dome of ERG unit was placed close to the eyes, and impedance and baseline tests programed on equipment were performed to decrease the noise of electrodes.
3) ERG procedure- After preparation of the dogs in ambient light, ERG was recorded following the diagnostic protocol for dogs recommended by ISCEV (14). To measure rod responses, 5 sets of 10 light stimuli (0.01 cd.s/m^2) at 2-second intervals were given, with a 4 minute delay between each set. The average of the ERG results of 10 light stimuli in each set was taken as the rod response figure, yielding a total of 5 different rod response records. It took about 20 minutes of dark adaptation to measure rod responses. Then the average of the results of 4 light stimuli (3 cd.s/m^2) at 10-second intervals was recorded as standard rod and cone response (Std R & C response). After 30 seconds of delay time, high-intensity rod and cone response (Hi-int R & C response) was recorded as the average of ERG evoked by 4 light flashes (10 cd.s/m^2) at 20-second intervals. After 10 minutes of background light adaptation (30 cd/m^2), cone response was recorded as the average of the results obtained by 32 flashes (3 cd.s/m^2) at 0.5-second intervals, followed by 30 Hz cone flicker response measurement with 128 flashes of the same light intensity under the background light adaptation of 30 cd/m^2.
4) Evaluation of ERG- The a-wave amplitude was measured from the baseline to the a-wave trough, and the b-wave amplitude from the a-wave trough to the b-wave peak. The a- and the b- wave implicit time were measured from the stimulus onset to the a-wave trough and the b-wave peak, respectively. The five rod responses obtained during 20 minutes of dark adaptation showed b-waves with a progressive rise in amplitude and decrease in implicit time as the frequency of flash increased. The last fifth response was chosen as the result of rod response because the fifth response was measured after the dog was adapted perfectly in dim light.

Dogs at diverse ages participated in this clinical trial, who were classified into three age groups: 1) 14 dogs (9 males, 5 females) under 5 years of age (group A), 2) 12 dogs (7 males, 5 females) between 5 and 10 years of age (group B), 3) 8 dogs (6 males, 2 females) over 10 years of age (group C).

Statistical analysis

All data were reported as mean value and standard deviation. Kruskal-Wallis test was used to compare mean values of a- and b-wave amplitudes and implicit time among three age groups. The difference between male and female was studied using Mann-Whitney U test. A value of $P < 0.05$ was considered significant for all comparisons. Data analysis was performed by use of computer software (SPSS 16.0 for window; SPSS Inc., Chicago, IL, U.S.A.).

Results

The waveform of typical normal ERG recorded from a beagle was shown in Fig 1. Rod responses had a prominent b-wave without a-wave that increased in amplitude and implicit time during the 20 min of dark adaptation. Std R & C response and Hi-int R & C response showed distinct a- and b-waves. Cone responses had smaller and faster a- and b-wave than other responses. Cone flicker response presented sequent b-waves.

Between Maltese and Shih Tzu, there were significant differences in the averages of a- and b- wave amplitude and implicit time of each response ($P < 0.05$), except for the implicit time of a- and b- wave of cone response and b- wave of cone flicker response.

In 20 eyes of 10 Maltese, the amplitudes (μV) of b-wave of rod, Std R & C, Hi-int R & C, cone, and flicker responses were 40.78 ± 23.42, 146.55 ± 65.34, 164.48 ± 99.19, 17.42 ± 7.29, and 21.14 ± 10.01. The amplitudes (μV) of a-wave of Std R & C, Hi-int R & C, and cone response were 44.29 ± 22, 47.46 ± 20.85, and 7.37 ± 5.34. The implicit time (ms) of b-wave of rod, Std R & C, Hi-int R & C, cone, and flicker responses were 118.59 ± 46.79, 76.47 ± 24.23, 63.36 ± 14.57, 36.66 ± 11.21, and 21.04 ± 7.68. The implicit time (ms) of a-

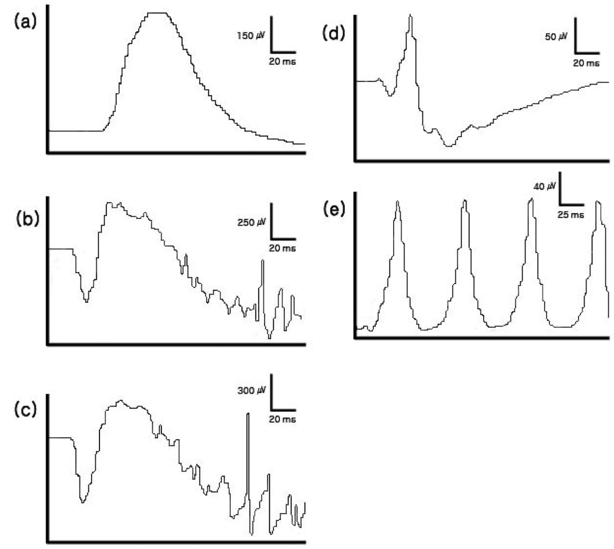

Fig 1. The waveform of 5 basic responses of ERG recording using HMsERG unit in a normal beagle dog. (a) rod response, (b) Standard rod and cone response, (c) Hi-intensity rod and cone response, (d) cone response, (e) flicker response.

wave of Std R & C, Hi-int R & C, and cone responses were 30.86 ± 21.8, 35.94 ± 23.08, and 21.27 ± 15.86.

In 14 eyes of 7 Shih Tzu, the amplitudes (µV) of b-wave of rod, Std R & C, Hi-int R & C, cone, and flicker responses were 53.02 ± 20.69, 141.22 ± 83, 137.58 ± 84, 29.77 ± 11.01, and 18.03 ± 7.96. The amplitudes (µV) of a-wave of Std R & C, Hi-int R & C, and cone responses were 39.75 ± 24.94, 40.93 ± 21.71, and 8.29 ± 5.46. The implicit time (ms) of b-wave of rod, Std R & C, Hi-int R & C, cone, and flicker responses were 99.45 ± 46.78, 81.04 ± 30.87, 68.74 ± 19.71, 45.94 ± 21.59, and 19.43 ± 6. The implicit time (ms) of a-wave of Std R & C, Hi-int R & C, and cone responses were 27.06 ± 19.78, 18.63 ± 4.99, and 16.42 ± 8.52.

Fig 2 showed the mean and standard deviation of amplitude (µV) of a-wave, implicit time (ms) of a-wave, amplitude (µV) of b-wave, implicit time (ms) of b-wave ERG in three age groups.

There were no significant differences in ERG responses between the age groups ($P > 0.05$), except for the implicit time of a-wave of Hi-int R & C response ($P < 0.05$).

The mean amplitude (µV) of b-wave of rod responses was 52.05 ± 38.54 in dogs under 5 years of age (group A), 45.28 ± 21.59 in dogs between 5 and 10 years of age (group B), and 55.87 ± 19.85 in dogs over 10 years of age (group C). The mean amplitude (µV) of a-wave of Std R & C response was 55.75 ± 36.17 in group A, 46.32 ± 17.26 in group B, and 50.03 ± 24.19 in group C. The mean amplitude (µV) of b-wave of Std R & C response was 140.16 ± 84.36 in group A, 143.8 ± 71.98 in group B, and 133.46 ± 65.01 in group C. The mean amplitude (µV) of a-wave of Hi-int R & C response was 49.51 ± 24.49 in group A, 51.13 ± 17.41 in group B, and 57.08 ± 23.53 in group C. The mean amplitude (µV) of b-wave of Hi-int R & C response was 179.35 ± 72.49 in group A, 160.92 ± 82.97 in group B, and 151.04 ± 96.48 in group C. The mean amplitude (µV) of a-wave of cone response was 9.41 ± 4.17 in group A, 9.2 ± 5.26 in group B, and 10.03 ± 6.36 in group C. The mean amplitude (µV) of b-wave of cone response was 35.55 ± 20.43 in group A, 29.31 ± 20.49 in group B, and 29.61 ± 13.29 in group C. The mean of amplitude (µV)

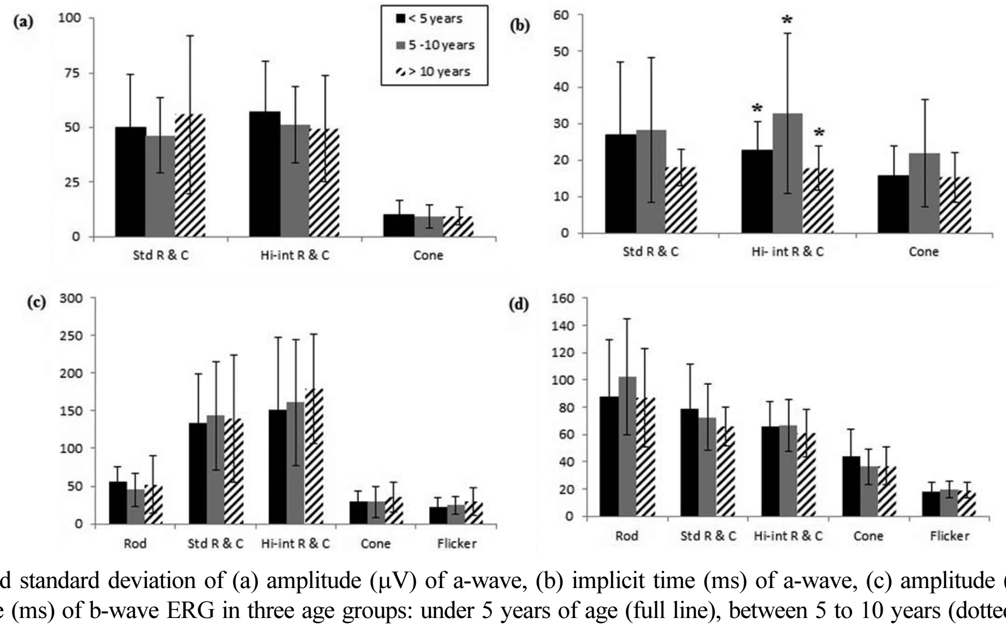

Fig 2. Mean and standard deviation of (a) amplitude (µV) of a-wave, (b) implicit time (ms) of a-wave, (c) amplitude (µV) of b-wave, (d) implicit time (ms) of b-wave ERG in three age groups: under 5 years of age (full line), between 5 to 10 years (dotted line), and over 10 years (broken line). The means are marked by black square in the group under 5 years of age, white square in the group between 5 to 10 years, and triangle in the group over 10 years. Each standard deviation is marked by horizontal bars and *indicates a significant difference between the three groups.

of b-wave of flicker response was 29.55 ± 18.58 in group A, 24.6 ± 12.08 in group B, and 22.26 ± 12.06 in group C.

The mean implicit time (ms) of b-wave of rod response was 86.76 ± 35.88 in group A, 102.48 ± 42.61 in group B, and 87.94 ± 41.41 in group C. The mean implicit time (ms) of a-wave of Std R & C response was 18.05 ± 4.93 in group A, 28.36 ± 19.82 in group B, and 26.95 ± 20.03 in group C. The mean implicit time (ms) of b-wave of Std R & C response was 65.87 ± 14.39 in group A, 72.6 ± 24.45 in group B, and 78.87 ± 33.13 in group C. The mean implicit time (ms) of a-wave of Hi-int R & C response was 17.75 ± 6.05 in group A, 32.94 ± 22.02 in group B, and 22.71 ± 7.81 in group C. The mean implicit time (ms) of b-wave of Hi-int R & C response was 61.11 ± 17.54 in group A, 66.8 ± 19.03 in group B, and 66.06 ± 18.33 in group C. The mean implicit time (ms) of a-wave of cone response was 15.29 ± 6.71 in group A, 21.95 ± 14.64 in group B, and 15.63 ± 8.63 in group C. The mean implicit time (ms) of b-wave of cone response was 37.24 ± 13.59 in group A, 36.45 ± 12.93 in group B, and 43.74 ± 19.87 in group C. The mean implicit time (ms) of b-wave of flicker response was 19.39 ± 5.74 in group A, 19.96 ± 6.22 in group B, and 18.43 ± 6.32 in group C.

Fig 3 showed the mean and standard deviation of amplitude (μV) of a-wave, implicit time (ms) of a-wave, amplitude (μV) of b-wave, implicit time (ms) of b-wave ERG between male group and female group.

There were no significant differences in ERG responses between male and female ($P > 0.05$), except for the implicit time of a-wave of cone response ($P < 0.05$).

The mean amplitude (μV) of b-wave of rod response was 54.21 ± 34 in male and 43.88 ± 16.88 in female. The mean amplitude (μV) of a-wave of Std R & C response was 54.34 ± 31.44 in male and 45.08 ± 18.97 in female. The mean amplitude (μV) of b-wave of Std R & C response was 155.69 ± 85.93 in male and 110.86 ± 34.25 in female. The mean amplitude (μV) of a-wave of Hi-int R & C response was 51.63 ± 22.99 in male and 52.28 ± 20.16 in female. The mean amplitude (μV) of b-wave of Hi-int R & C response was 178.47 ± 88.24 in male and 143.66 ± 64.4 in female. The mean amplitude (μV) of a-wave of cone response was 8.94 ± 5.15 in male and 10.47 ± 4.87 in female. The mean amplitude (μV) of b-wave of cone response was 29.25 ± 18.05 in male and 36.9 ± 20.09 in female. The mean amplitude (μV) of b-wave of flicker response was 24.79 ± 16.35 in male and 28.47 ± 12.8 in female.

The mean implicit time (ms) of b-wave of rod response was 96.21 ± 40.62 in male and 85.93 ± 38.05 in female. The mean implicit time (ms) of a-wave of Std R & C response was 25.37 ± 19.48 in male and 20.88 ± 5.41 in female. The mean implicit time (ms) of b-wave of Std R & C response was 73.65 ± 26.51 in male and 67 ± 17.03 in female. The mean implicit time (ms) of a-wave of Hi-int R & C response was 25.84 ± 18.41 in male and 21.42 ± 7.29 in female. The mean implicit time (ms) of b-wave of Hi-int R & C response was 63.85 ± 18.77 in male and 65.07 ± 17.42 in female. The mean implicit time (ms) of a-wave of cone response was 15.67 ± 8.94 in male and 21.48 ± 13 in female. The mean implicit time (ms) of b-wave of cone response was 37.41 ± 15.82 in male and 40.46 ± 13.87 in female. The mean implicit time (ms) of b-wave of flicker response was 19.65 ± 5.84 in male and 18.55 ± 6.29 in female.

Discussion

Compared with ERG values in Shih Tzu dog reported previously by our laboratory (11), the results of this study

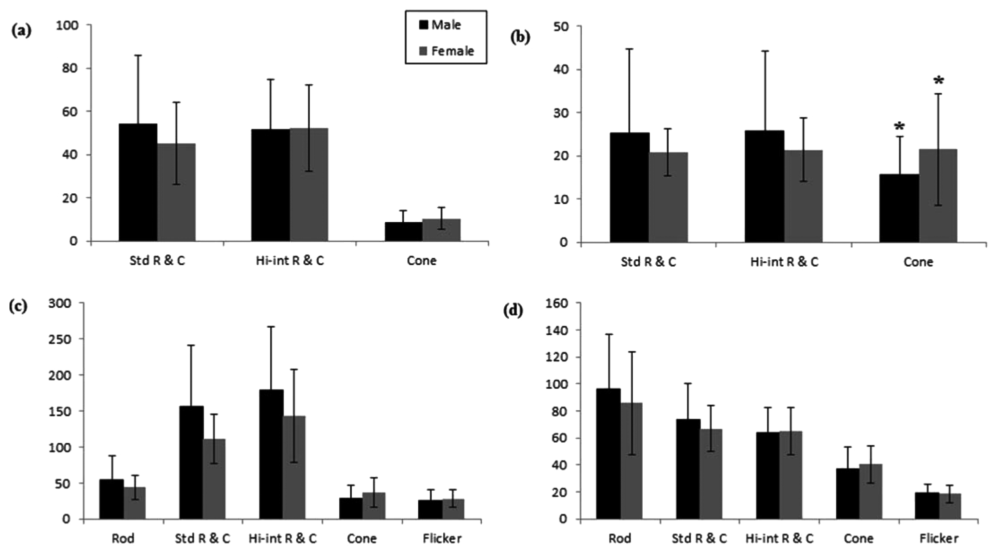

Fig 3. Mean and standard deviation of (a) amplitude (μV) of a-wave, (b) implicit time (ms) of a-wave, (c) amplitude (μV) of b-wave, (d) implicit time (ms) of b-wave ERG between male group (full line) and female group (dotted line). The means are marked by black square in male group, and white square in female group. Each standard deviation is marked by horizontal bars and *indicates a significant difference between genders.

showed generally lower values. The biggest difference between the two studies lied in anesthetic protocol. Combination of medetomidine and tiletamine-zolazepam was used as anesthetics in this study, while combination of medetomidine and ketamine was the anesthetics used in the previous study. Further studies are needed on the specific cause and effect of these anesthetics on ERG results.

In human, many studies reported on the correlation of age and gender with ERG results, while less attention was given on the difference between races (2,5,7,12). However, the difference among breeds may act as a dominant affecting factor in ERG results in dogs. In this study, there were significant differences in the averages of a- and b- wave amplitude and implicit time of each response between Maltese and Shih Tzu, except for the implicit time of a- and b- wave of cone response and b- wave of cone flicker response. The gold rim of contact electrode known to act as artificial pupil and adjust the amount of light may not be suitable in some breeds in dogs because the depth of the orbit shows quite a difference between breeds due to the diversity of the size of canine skull. Moreover, contact lens electrode that measured up to the corneal curvature of human did not fit perfectly in all breeds of dogs (10).

In human, many studies reported on the decrease of amplitude and the delay of implicit time in b-wave according to aging (2,12). The cause of this tendency was considered as the diminished retinal illuminance, and slight reduction in sensitivity due to the deposit of lipofuscin in retinal epithelial cell that leaded to the death of photoreceptors (2). However, this study did not obtain remarkable result related to aging, possibly due to the limitation that this clinical trial did not control ages of the objects. The effect of aging on ERG result should be further studied in the continuing research with detailed classification of ages.

In this study, no significant difference was found in amplitude and implicit time of ERG result between male and female, except for the implicit time of a-wave of cone response. However, significant difference in amplitude between genders was reported previously in human (1). One previous study reported that the axial lengths of eyeballs were greater in males than in females, which led to smaller amplitudes in males (15,20).

In this study, the oscillatory potential thought to reflect electrical activity initiated by amacrine cells in the inner retina was not measured because of the wide variation in the results of different laboratories (1). Instead of the oscillatory potential, we included the Hi-int rod & cone response, which was obtained in the dark-adapted condition.

ISCEV recommended that each laboratory establish the standardization of ERG procedure including anesthetic protocol and measurement of normal value of dogs with its own equipment in a particular facility (14). ERG is helpful for young dogs in need of an early diagnosis of inherited retinal disease, and for the candidates for cataract surgery as it contributes to prediction of prognosis by confirming subclinical retinopathy. The protocol and results of this study may serve as a guideline for other ERG studies of dogs.

Acknowledgements

This paper was supported by Konkuk University in 2010.

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건강한 내원견에서 나이와 성별이 망막전위도에 미치는 영향

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요 약 : 본 연구의 목적은 건강한 내원견을 대상으로 측정한 망막전위도에 나이 및 성별이 어떠한 영향을 미치는지 알아보는데 있다. 보호자가 임상 실험에 참가하는 것을 동의한 34두의 개를 medetomidine과 tiletamine-zolazepam혼합제로 전신마취하여 ISCEV에서 권고하는 진단용 프로토콜에 따라 망막전위도를 측정하였다. 성별간 비교시는 cone 반응의 a파의 지연시간에서 그리고 나이별 비교시 Hi-int R & C 반응의 a파 지연시간에서 유의성을 각각 나타내었다 ($P < 0.05$). 나머지 ERG반응들은 나이와 성별에 영향을 받지 않는 것으로 나타났다.

주요어 : 건강견, 망막전위도, 나이, 성별