The Journal of Veterinary Medical Science

NOTE

Surgery

Effects of pupil size on canine visual evoked potential with pattern stimulation

Seiya MAEHARA[1)]*, Yoshiki ITOH[2)], Wataru KURIMOTO[1)], Yasunari KITAMURA[3)], Yosuke ITO[1)], Miri HAYASHI[1)] and Arisa MASUKO[1)]

[1)]Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan
[2)]Department of Veterinary Ophthalmology, Faculty of Veterinary Medicine, Okayama University of Science, 1-3 Ikoinooka, Imabari, Ehime, 794-8555, Japan
[3)]Yakumo Animal Hospital, 91, Shinonome-cho, Yakumo-cho, Hokkaido 049-3105, Japan

J. Vet. Med. Sci.
82(7): 922–925, 2020
doi: 10.1292/jvms.20-0169

Received: 23 March 2020
Accepted: 9 May 2020
Advanced Epub: 21 May 2020

ABSTRACT. The purpose of this study was to investigate the effects of pupil diameter on canine visual evoked potentials with pattern stimulation (P-VEP). Atropine eye drop (1.0%) was applied to both eyes as a cycloplegic drug, and tafluprost eye drop (0.015%) was applied to one eye that was selected randomly for miosis (miosis group). The other eye did not receive tafluprost (mydriasis group). P-VEP was recorded at three pattern sizes. The P100 implicit time at a small pattern size in the mydriasis group was significantly prolonged compared to the miosis group. We hypothesized that the prolonged P100 implicit time under mydriatic conditions was due to increased spherical aberrations and concluded that mydriatic conditions affected P100 implicit time in canine P-VEP recordings.

KEY WORDS: canine, spherical aberration, visual evoked potential with pattern stimulation

The visual evoked potential (VEP) test is an examination that can objectively evaluate visual acuity. The method works detecting brain wave signals from the visual cortex that are induced by a light stimulus [5, 6]. VEP is classified into flash-stimulated VEP (F-VEP), which uses a flash stimulus, and pattern-stimulated VEP (P-VEP), which uses a contrast-reversing checkerboard pattern stimulus. In our previous studies, the effects of the refractive power of the eye [10] and anesthesia [9] on canine P-VEP were reported, and we measured canine visual acuity using P-VEP [17].

The refractive power of the eye affects P100 implicit time in canine P-VEP recordings, and P-VEP is recorded stably when this power is adjusted to a stimulus monitor [10]. To measure the refractive power of the eye, cycloplegic drugs such as atropine or cyclopentrate [10, 11, 16]. However, since cycloplegic drugs are cholinergic antagonists, pupillary dilation also occurs [8]. As the pupil dilates, the effective diameter of the lens increases, and spherical aberrations also increase [4, 7, 15, 18, 20].

Spherical aberration is a phenomenon in which the focus position of the light differs due to differences in the incident angle, refraction angle, and optical path length of paraxial rays that pass near the optical axis of the lens, and peripheral rays that pass away from the optical axis [4, 7, 10, 18, 20]. Because increasing spherical aberration reduces visual perception due to the differing focus positions of light on the retina, P-VEP is also affected. In this study, P-VEPs with different pupil diameters were recorded to investigate the effects of pupil diameter on canine P-VEP.

Twelve eyes from six clinically normal beagle dogs (3 males and 3 females) were used in this study. The dogs were 3 to 4 years of age and weighed 11.1 to 14.4 kg. These animals showed no abnormalities in ophthalmic examinations before the study. The examinations included pupillary light reflex, dazzle reflex, menace response, applanation tonometry (Tono-Pen XL, Medtronic Solan, Jacksonville, FL, USA), slit-lamp biomicroscopy (SL-7, Kowa, Nagoya, Japan), ophthalmoscopy (TRC-50IX, TOPCON, Tokyo, Japan) and electroretinography (LE-3000, TOMEY, Nagoya, Japan). This study was conducted in accordance with the guidelines of Experimental Animal Research Committee of Rakuno Gakuen University and was approved by the committee (No. VH17B21).

A single drop of atropine sulfate eye drop (Nitten ATROPINE Ophthalmic Solution 1%, Nitten Pharmaceutical Co., Ltd., Nagoya, Japan) was applied to both eyes as a cycloplegic and mydriatic drug. Thirty minutes later, a single drop of tafluprost (TAPROS ophthalmic solution 0.0015%, Santen Pharmaceutical Co., Ltd., Osaka, Japan) eye drop was applied to one eye that was randomly selected for miosis (miosis group) and was not applied to the other eye (mydriasis group). The refractive power of each recorded eye was measured using skiascopy in accordance with our previous report [16]. Refractive power was measured with a streak retinoscope (Streak Retinoscope RX-3A, Neits Instruments Co., Ltd., Tokyo, Japan) and a skiascopic lens (Hatake Skiascope, Handaya Co., Ltd., Tokyo, Japan) under dim light 90 min after applying atropine eye drops. The refractive power of

*Correspondence to: Maehara, S.: seiya-m@rakuno.ac.jp

each recorded eye was corrected to −2 diopters according to a testing distance of 0.5 m using soft contact lenses (Premio, Menicon, Nagoya, Japan) based on the obtained skiascopy data.

Prior to VEP recording, the pupil diameter of the eye was measured. VEP were recorded under sedation using a combination of 0.01 mg/kg medetomidine (Domitor, ZENOAQ, Fukushima, Japan), 0.15 mg/kg midazolam (Dormicam, Astellas Pharma, Tokyo, Japan) and 0.025 mg/kg butorphanol (Vetorphale, Meiji Seika Pharma, Tokyo, Japan) injected intravenously. A portable ERG/VEP system and pattern stimulus display (PS-410, TOMEY) were used for this study. The details of this display were as follows: indicated color, yellow (580 nm); resolution, 640 × 400 dots; indicated area, 122 × 195 mm; pixel size, 0.22 × 0.22 mm; frame frequency, 60 Hz; contrast, 75%; and mean luminosity, 15 cd/m^2. The stimulus monitor was placed 0.5 m in front of the eye, and three stimulus pattern sizes were used. The length of one side of each square pattern was 31.72 (No. 1), 7.31 (No. 2) and 1.22 mm (No. 3). For P-VEP recordings, needle electrodes (VEP needle electrodes, Mayo Corp., Nagoya, Japan) were positioned at the inion (external occipital protuberance) for the recording electrode and at the nasion (nasal point) for the reference electrode. A plate-type electrode (LE ear electrode, Mayo Corp.) was positioned on the inner surface of the right auricle as an earth electrode, in accordance with previous reports [9, 10, 17]. During VEP recordings, the eyelid was retracted with a speculum, and a supporting thread was placed in the dorsal conjunctiva using 6–0 silk (MANI, Utsunomiya, Japan) to fix the eye position. The VEP was recorded for each eye, and the eye that did not record VEP was shielded with a hand. The stimulation rate was 3 reversals/sec, and the P-VEP signal was averaged from 128 repetitions.

The P100 implicit time and N75-P100 amplitude were estimated according to a standard determined by the International Society for Clinical Electrophysiology of Vision [19]. The P100 implicit time and N75-P100 amplitude recorded for each pattern size were compared between the miosis and mydriasis group using a Student's *t*-test. The P100 implicit time and N75-P100 amplitude recorded each testing pattern size in each group were compared using one-way factorial analysis of variance (ANOVA) with Fisher's PLSD test. The statistical significance of differences was determined with $P<0.05$ as the minimum level of acceptable significance.

The pupil diameter before VEP recording in the miosis and mydriasis groups were 1.1 ± 0.4 (mean ± SD) and 10.2 ± 0.9 mm, respectively. Photographs of one eye in each group are shown in Fig. 1.

The typical VEP waveforms obtained for one dog are shown in Fig. 2. The P100 implicit time and N75-P100 amplitude in each group are shown in Tables 1 and 2, respectively. The P100 implicit time in the mydriasis group was significantly prolonged

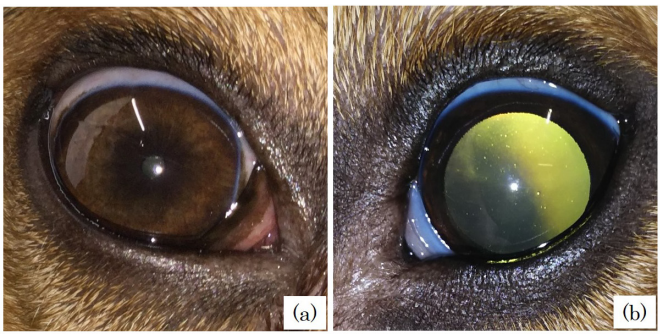

Fig. 1. Photographs of the eyes in the miosis group (a) and myrdriasis group (b).

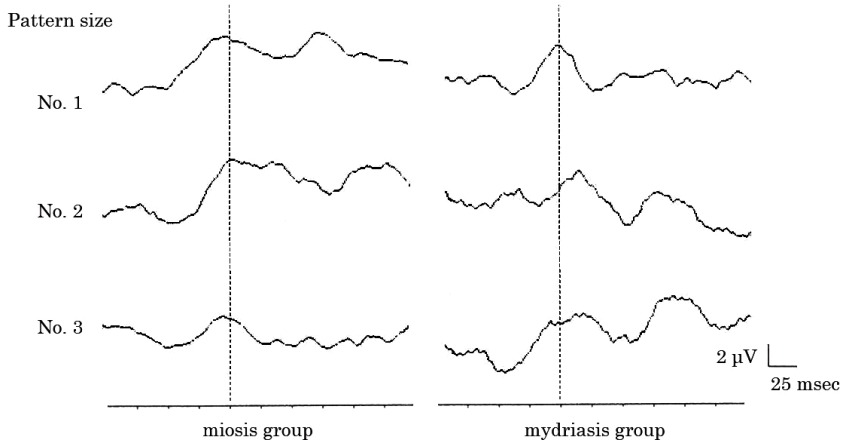

Fig. 2. Visual evoked potential with pattern stimulus waveforms obtained from one dog at each pattern size. The dotted lines indicate 100 msec after stimulation.

Table 1. The P100 implicit time in the miosis and mydriasis group

Group	Pattern size (msec)		
	No. 1	No. 2 [a,b)]	No. 3 [a,b)]
Miosis	102.7 ± 12.5	101.4 ± 13.6	104.5 ± 13.2
Mydriasis	101.9 ± 13.1	119.4 ± 19.4	118.6 ± 19.9

a) Significant difference between the two groups (P<0.05). b) Significantly different from pattern size No. 1 in the mydriasis group.

Table 2. The N75-P100 amplitude in the miosis and mydriasis group

Group	Pattern size (μV)		
	No. 1	No. 2	No. 3
Miosis	3.7 ± 1.6	3.5 ± 1.6	3.4 ± 1.5
Mydriasis	3.6 ± 1.6	3.7 ± 1.5	3.8 ± 1.5

compared to the miosis group at pattern sizes Nos. 2 and 3 (P<0.05). In the miosis group, there was no significant difference between pattern sizes, while in the mydriasis group, the P100 implicit time at Nos. 2 and 3 was significantly prolonged compared to No. 1 (P<0.05). There was no significant difference in the N75-P100 amplitude between the two groups and between pattern sizes in each group.

In the present study, P-VEPs in miotic and mydriatic conditions were recorded, and the waveforms were compared. Under mydriatic conditions, prolongation of P100 implicit time was detected. This result was likely due to decreased visual recognition resulting from increased spherical aberration under mydriatic conditions.

In this study, tafluprost eye drops were used to obtain miosis conditions. Tafluprost is a prostaglandin analogues used in glaucoma therapy [21]. The stimulation of miosis in dogs by prostaglandins is thought to occur by direct action on prostaglandin receptor located on the iridal sphincter muscle [23]. Generally, the cholinergic agonist pilocarpine is a miotic drug used in dogs. However, it has been reported that pilocarpine eye drops can cause uveitis due to irritation when applied [13]. The influence of uveitis on refraction and P-VEP has not been reported. Therefore, tafluprost, which is a prostaglandin analogue, was used instead of pilocarpine to obtain miosis conditions in this study.

Prolongation of the P100 implicit time decreases visual recognition. In human medical science, prolonged P100 implicit times has been observed in patients with optic neuritis [2]. In dogs, prolongation of the P100 implicit time has been reported when the refractive power of the eye is not adjusted to the distance of the stimulus pattern monitor [10]. In the miotic condition in this study, P100 implicit time was observed around 100 msec after stimulation for all pattern sizes, while under mydriatic conditions, the P100 implicit time was prolonged at the small pattern size. This suggests that visual recognition was decreased under mydriatic conditions compared with miotic conditions.

We suspect that prolongation of the P100 implicit time in mydriatic conditions, that is, the decrease in visual recognition, was due to an increase in spherical aberration. Aberration refer to a phenomenon in which the position of the focusing light differs depending on the wavelength of light, and the position and direction of the passing lens [4, 7, 18, 20]. Monochromatic aberrations include coma, astigmatism, field curvature, distortion, and spherical aberration, and these are called Seidel's five aberrations. Spherical aberration is a phenomenon in which the focus position of the light differs as a result of differences in incident angle, refraction angle, and optical path length of paraxial rays that pass near the optical axis of the lens, and peripheral rays that pass away from the optical axis [4, 7, 14, 18, 20]. In humans, it has been reported that a reduction in pupil diameter reduces spherical aberration and improves retinal imaging [12, 22]. Heilman *et al.* also reported that peripheral rays were more challenging to focus on than paraxial rays in cynomolgus monkey lens [15]. In the mydriasis group in our study, we hypothesize that the effective diameter of the lens was increased and spherical aberration was increased due to pupil dilation, and this resulted in a prolonged P100 implicit time compared to the miosis group.

Alternatively, the possibility of diffraction has to be considered in the miotic eye. Diffraction is the phenomenon where light rays passing through the edge of an opaque object go behind obstacles [4, 7]. Diffraction can also lead to a decrease in visual recognition. However, the P100 implicit time in the miosis group in our study was almost 100 msec after stimulation. We believe that diffraction caused by miosis has a small effect on recording P-VEP in dogs. The limitation of this study is that our P-VEP data were recorded from eyes with extremely different pupil conditions. In future studies, it will be necessary to compare VEPs obtained from eyes with various pupil sizes, rather than miotic and mydriatic eyes. The other limitation of this study was that our data were obtained from only young dogs. The lens state is changed by aging; for example, lenses develop nuclear sclerosis with aging [3], and the refractive value of a lens changes due to nuclear sclerosis [16]. Changes in the refraction of the lens may affect spherical aberration, and therefore age-related changes should be investigated in future studies.

There was no significant difference in the N75-P100 amplitude between the two groups and pattern sizes in each group. It has been reported that many factors (e.g., illumination in the laboratory room, the condition of the optic media, fixation to the stimulus device, and drowsiness) affect the N75-P100 amplitude in human P-VEP recording [1]. In previous reports on canine P-VEP, it was difficult to evaluate the N75-P100 amplitude as a result of variation [9, 10]. As in previous reports, the N75-P100 amplitude had a large standard deviation and individual differences in the present study.

From the results of this study, we suggest that it is necessary to consider pupil size when recording and evaluating canine P-VEPs, and note that P100 implicit time may be prolonged, especially under mydriatic conditions.

REFERENCES

1. Brigell, M. G. 2001. The visual evoked potential. pp. 237–279. *In*: Electrophysiologic Testing, 2nd ed. (Fishman, G. A., Birch, D. G., Holder, G. E. and Brigell, M. G. eds.), The Foundation of the American Academy of Ophthalmology, San Francisco.
2. Chatziralli, I. P., Moschos, M. M., Brouzas, D., Kopsidas, K. and Ladas, I. D. 2012. Evaluation of retinal nerve fibre layer thickness and visual evoked potentials in optic neuritis associated with multiple sclerosis. *Clin. Exp. Optom.* **95**: 223–228. [Medline] [CrossRef]
3. Davidoson, M. G. and Nelms, S. R. 2013. Diseases of the lens and cataract formation. pp. 1199–1233. *In*: Veterinary Ophthalmology, 5th ed. (Gelatt, K. N., Gilger, B. C. and Kern, T. J. eds.), John Wiley & Sons Inc, Ames.
4. Davson, H. 1990. Davson's Physiology of the Eye, 5th ed., Macmillan Press, London.
5. Ekesten, B. 2007. Ophthalmic examination and diagnostics. Part 4: electrodiagnostic evaluation of vision. pp. 520–535. *In*: Veterinary Ophthalmology, 4th ed. (Gelatt, K. N. ed.), Blackwell Publishing, Oxford.
6. Fahle, M. and Bach, M. 2006. Origin of the visual evoked potentials. pp. 207–234. *In*: Principles and Practice of Clinical Electrophysiology of Vision, 2nd ed. (John, R. H. and Geoffrey, B. A. eds.), The MIT Press, London.
7. Green, G. G. 1994. Visual acuity, color vision, and adaptation. pp. 332–349. *In*: Principles and Practice of Ophthalmology (Albert, D. M. and Jakobiec, F. A. eds.), W. B. Saunders, Philadelphia.
8. Herring, I. P. 2013. Clinical pharmacology and therapeutics. Part 4: mydriatics/cycloplegics, anesthetics, and tear substitutes and stimulators. pp. 423–434. *In*: Veterinary Ophthalmology, 5th ed. (Gelatt, K. N., Gilger, B. C. and Kern, T. J. eds.), John Wiley & Sons Inc., Ames.
9. Ito, Y., Maehara, S., Itoh, Y., Hayashi, M., Kubo, A., Itami, T., Ishizuka, T., Tamura, J. and Yamashita, K. 2015. Effect of sevoflurane concentration on visual evoked potentials with pattern stimulation in dogs. *J. Vet. Med. Sci.* **77**: 155–160. [Medline] [CrossRef]
10. Ito, Y., Maehara, S., Itoh, Y., Matsui, A., Hayashi, M., Kubo, A. and Uchide, T. 2016. Effect of refractive error on visual evoked potentials with pattern stimulation in dogs. *J. Vet. Med. Sci.* **78**: 505–508. [Medline] [CrossRef]
11. Itoh, Y., Hagiwara, M., Maehara, S. and Izumisawa, Y. 2011. Accuracy of hand-held autorefractometer for refractive examination of dog's eye, and influence of accommodative palsy. *Anim. Eye Res.* **30**: 3–10. [CrossRef]
12. Kawamorita, T., Yamamoto, S., Nakayama, N. and Uozato, H. 2007. Temporal changes in pupil diameter and wavefront higher-order aberration after blinking. *Jpn. J. Vis. Res.* **28**: 168–171.
13. Krohne, S. G., Gionfriddo, J. and Morrison, E. A. 1998. Inhibition of pilocarpine-induced aqueous humor flare, hypotony, and miosis by topical administration of anti-inflammatory and anesthetic drugs to dogs. *Am. J. Vet. Res.* **59**: 482–488. [Medline]
14. López-Gil, N., Peixoto-de-Matos, S. C., Thibos, L. N. and González-Méijome, J. M. 2012. Shedding light on night myopia. *J. Vis.* **12**: 4. [Medline] [CrossRef]
15. Maceo Heilman, B., Manns, F., de Castro, A., Durkee, H., Arrieta, E., Marcos, S. and Parel, J. M. 2015. Changes in monkey crystalline lens spherical aberration during simulated accommodation in a lens stretcher. *Invest. Ophthalmol. Vis. Sci.* **56**: 1743–1750. [Medline] [CrossRef]
16. Maehara, S., Itoh, Y., Higashinozono, K. and Izumisawa, Y. 2011. Evaluation of refractive value by skiascopy in healthy Beagles. *J. Vet. Med. Sci.* **73**: 927–929. [Medline] [CrossRef]
17. Maehara, S., Itoh, Y., Ito, Y., Hayashi, M. and Masuko, A. 2018. Measurement of visual acuity in Beagle dog by visual evoked potential with pattern stimulation. *J. Vet. Med. Sci.* **80**: 1758–1761. [Medline] [CrossRef]
18. Marín-Franch, I., Xu, R., Bradley, A., Thibos, L. N. and López-Gil, N. 2018. The effect of spherical aberration on visual performance and refractive state for stimuli and tasks typical of night viewing. *J. Optom.* **11**: 144–152. [Medline] [CrossRef]
19. Odom, J. V., Bach, M., Brigell, M., Holder, G. E., McCulloch, D. L., Tormene, A. P. and Vaegan. 2010. ISCEV standard for clinical visual evoked potentials (2009 update). *Doc. Ophthalmol.* **120**: 111–119. [Medline] [CrossRef]
20. Ofri, R. 2013. Optics and physiology of vision. pp. 208–270. *In*: Veterinary Ophthalmology, 5th ed. (Gelatt, K. N., Gilger, B. C. and Kern, T. J. eds.), John Wiley & Sons Inc., Ames.
21. Plummer, C. E., Regnier, A. and Gelatt, K. N. 2013. The canine glaucomas. pp. 1050–1145. *In*: Veterinary Ophthalmology, 5th ed. (Gelatt, K. N., Gilger, B. C. and Kern, T. J. eds.), John Wiley & Sons Inc., Ames.
22. Yamamoto, S., Kawamorita, T., Nakayama, N. and Uozato, H. 2007. Effect of blinking on pupil diameter and objective refraction. *Jpn. J. Vis. Res.* **28**: 162–167.
23. Yoshitomi, T. and Ito, Y. 1988. Effects of indomethacin and prostaglandins on the dog iris sphincter and dilator muscles. *Invest. Ophthalmol. Vis. Sci.* **29**: 127–132. [Medline]