NOTE *Internal Medicine*

Effect of refractive error on visual evoked potentials with pattern stimulation in dogs

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ABSTRACT. The purpose of this study was to investigate the effects of refractive error on canine visual evoked potentials with pattern stimulation (P-VEP). Six normal beagle dogs were used. The refractive power of the recorded eyes was measured by skiascopy. The refractive power was corrected to −4 diopters (D) to +2 D using contact lens. P-VEP was recorded at each refractive power. The stimulus pattern size and distance were 50.3 arc-min and 50 cm. The P100 appeared at almost 100 msec at −2 D (at which the stimulus monitor was in focus). There was significant prolongation of the P100 implicit time at −4, −3, 0 and +1 D compared with −2 D, respectively. We concluded that the refractive power of the eye affected the P100 implicit time in canine P-VEP recording.
KEY WORDS: canine, P100 implicit time, pattern stimulated visual evoked potential, refractive power

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The visual evoked potential (VEP) test is a method for detecting the brain wave signals from the visual cortex induced by a light stimulus. VEP is affected by the function of all regions in the visual pathway and classified into pattern-stimulated VEP (P-VEP) with a contrast-reversing checkerboard pattern stimulus and flash-stimulated VEP (F-VEP) with a flash stimulus. In human medicine, the F-VEP test is useful when a subject has poor vision and/or cooperates poorly (e.g., infants, children). The P-VEP is used to support the diagnosis of a nervous abnormality (e.g., optic neuritis, multiple sclerosis) or a psychogenic visual disturbance or malingering [10, 14]. However, cooperation of the subject is necessary to perform the P-VEP test. Moreover, the P-VEP test is applied as a visual acuity test [7, 11, 15].

In the veterinary medical science, the VEP test is used to evaluate development and neurologic disorders of visual function in postnatal animals and to investigate toxic effects on the visual pathway in toxicological tests [12]. There are some reports indicating that the VEP test may be useful for evaluation of the visual pathway clinically, as in the case of human medicine. There were some reports indicating a relation between the P-VEP and stimulus pattern size or depth of anesthesia [5, 6]. However, there are few studies that have performed steady-state P-VEP in consideration of refractive power; on the other hand, no studies have performed transient P-VEP.

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In this study, we recorded P-VEP with a constant stimulus pattern size and stimulus distance and with changing refractive power of the eye to investigate the effects of refractive error of the eye on P-VEP.

Six eyes from six clinically normal beagle dogs (four males and two females) were used in this study. The dogs were 4 to 6 years of age (mean: 5.8 years) and weighed 10.7 to 14.5 kg (mean: 13.9 kg). They had no abnormalities in neurologic and ophthalmic examinations before the study. Examinations included pupillary light reflex, menace response, applanation tonometry (Tono-Pen XL, Medtronic Solan, Jacksonville, FL, U.S.A.), 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 according to the guidelines of the Experimental Animal Research Committee of Rakuno Gakuen University.

The refractive power of the recorded eyes was measured by skiascopy according to the previous method reported by Maehara *et al*. [8]. The 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 60 min after applying cyclopentolate hydrochloride eye drops (Cyplegin 1%, Santen Pharmaceutical Co., Ltd., Osaka, Japan) as a cycloplegic drug. The measurement distance was set as 50 cm. The refractive power of the eyes was corrected to −4 diopters (D), −3 D, −2 D, −1 D, 0 D, +1 D and +2 D using soft contact lenses (SCLs; PremiO, Menicon Co., Ltd., Nagoya, Japan) after obtaining the results of the skiascopy. The refractive power of the subject's eye was remeasured by skiascopy to confirm that the refractive power was corrected. For P-VEP recording, needle electrodes (VEP needle electrodes, Mayo Corporation, Nagoya, Japan) were positioned at the inion (external occipital pro-

tuberance) as the recording electrode and the nasion (nasal point) as the reference electrode. A plate-type electrode (LE ear electrode, Mayo Corporation) was positioned on the inner surface of the right auricle as an earth electrode, as in previous reports [5, 6]. Prior to recording, all dogs were sedated with 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) injected intravenously. P-VEP was recorded with a portable VEP system (LE-3000, Tomey) and pattern stimulus display (PS-410, Tomey). 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 testing distance, which was the distance from the cornea to the display, was 50 cm, the stimulus pattern size was 7.31 mm, and the visual angle was 50.3 arc-min at −2 D, which represented perfect focus. This visual angle was adopted based on a previous study [6]. The stimulation rate was 3 reversals/sec. The P-VEP signal was averaged from 64 repetitions. P-VEP was recorded under dim lighting. An eye speculum (BARRAQUER Speculum, Inami & Co., Ltd., Tokyo, Japan) was used to keep the eyelids open, and physiological saline eye drops were instilled for corneal hydration. The subject's eye was supported with a 6-0 silk thread (6-0 Silk, MANI, Utsunomiya, Japan) through the dorsal bulbar conjunctiva to gaze at the pattern stimulus display. The unrecorded eye was occluded with a bandage to avoid stimulation.

In P-VEP recording in humans, the largest negative peak appears at almost 75 msec and is referred to as the N75 after stimulation, and the largest positive peak appears at almost 100 msec and is referred to as the P100 [2]. In this study, P100 implicit time and N75-P100 amplitude were estimated according to a standard determined by the International Society for Clinical Electrophysiology of Vision (ISCEV) [13]. In each dog, P-VEP recording was performed three times, and the mean was calculated from the data of the individual. The P100 implicit times and N75-P100 amplitudes obtained at −2 D were compared with the P100 implicit times and N75-P100 amplitudes obtained at all other test refractive powers by paired *t*-test. The statistical significance of differences was determined with $P<0.05$ as the minimum level of acceptable significance.

The refractive power of the eye is shown for each dog in Table 1. Typical P-VEP waveforms obtained from dog No.6 at each refractive power are shown in Fig. 1. Graphs for the P100 implicit time and refractive power and the N-75-P100 amplitude and refractive power are shown in Figs. 2 and 3, respectively. The P100 implicit time was 127.9 ± 12.7 msec (mean ± SD) at −4 D, 118.7 ± 14.6 msec at −3 D, 101.2 ± 2.5 msec at −2 D, 115.6 ± 21.3 msec at −1 D, 108.0 ± 17.2 msec at 0 D, 125.9 ± 14.4 msec at +1 D and 120.1 ± 19.2 msec at +2 D. There were significant increases in P100 implicit time between −2 D and −4 (P=0.004), −3 (P=0.004) and +1 D (P=0.003), respectively. The N75-P100 amplitude was 2.0 ± 0.6 (mean ± SD) μV at −4 D, 2.0 ± 0.8 μV at −3 D, 2.1 ± 0.4 μV at −2 D, 2.5 ± 1.4 μV at −1 D, 2.6 ± 0.7 μV at 0 D, 2.2 ± 1.3 μV at +1 D and 2.6 ± 1.0 μV at +2 D. There was a significant difference between the N75-P100 amplitude at −2 D and that at 0 D (P=0.004).

Table 1. The refractive power of the eye for each dog

Dog No.	Refractive power of eye
1	+1.5
2	+1.0
3	+1.5
4	+1.5
5	+2.0
6	+0.5

Refractive power is shown in diopters.

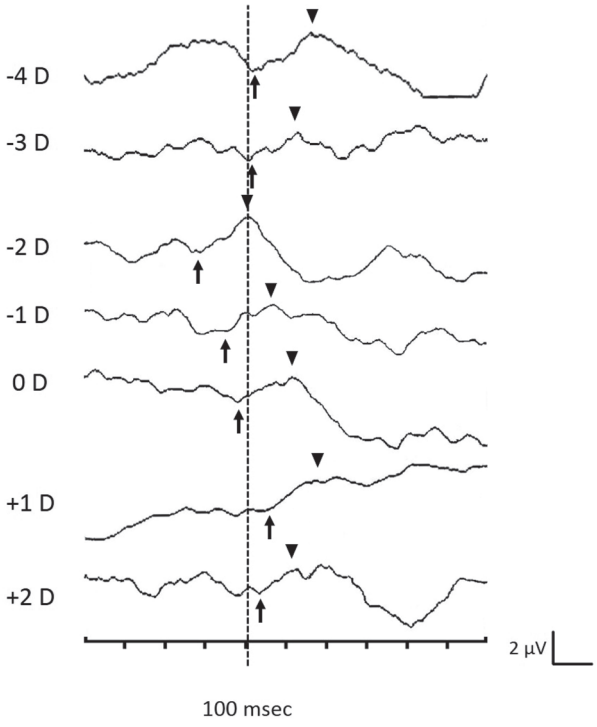

Fig. 1. P-VEP waveforms obtained from dog No. 6 at each refractive power. Arrows and arrowheads indicate the N75 and P100, respectively.

In this study, the P100 implicit time was recorded stably at almost 100 msec at −2 D, with the standard deviation being very small. However, at −4, −3 and +1 D, the P100 implicit time was prolonged. Therefore, it was suggested that the refractive power of the eye affected the P100 implicit time in P-VEP recording in the present study.

Ametropia is classified by refractive power. Emmetropia is the state in which light is focused on the retina, myopia is the state in which light is focused on the front of the retina, and hyperopia is the state in which light is focused behind the retina. Therefore, focal correction of ametropia is necessary to recognize an object clearly in a myopic or hyperopic eye.

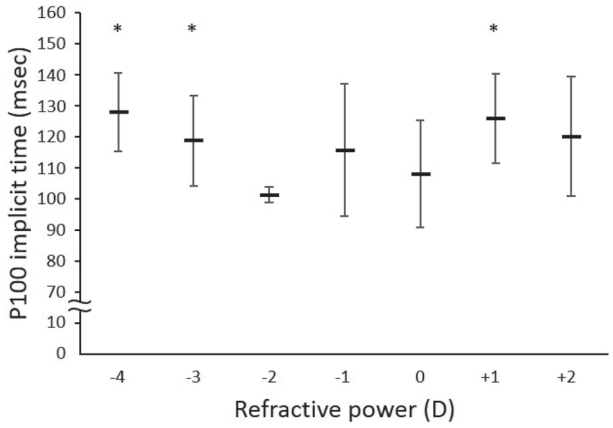

Fig. 2. The graph shows the correlation between the P100 implicit time and different refractive powers of the eye. The bars show the means at each refractive power. Significant differences compared with −2 D are shown by asterisks.

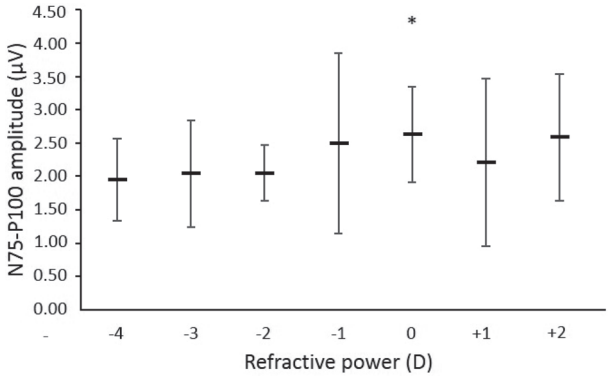

Fig. 3. The graph shows the correlation between the N75-P100 amplitude and different refractive powers of the eye. The bars show the means at each refractive power. A significant difference compared with −2 D is shown by the asterisk.

In this study, we performed P-VEP recording after administration cycloplegic eye drops and set the distance from the cornea to the display as 50 cm. A subject with the refractive power of the eye corrected to −2 D could recognize clearly a pattern stimulus display that was set 50 cm from the eye being recorded. The P100 appeared at almost 100 msec in this study, but only when recording P-VEP at −2 D. The P100 is a positive peak of the P-VEP occurring about 100 msec after stimulus in humans [2]. Similarly, it is reported that the P100 appears at around 100 msec when a subject recognizes a stimulus pattern well in dogs [6]. It seems that only the recorded eyes corrected as −2 D by a SCL could recognize clearly a stimulus pattern projected on a stimulus display.

The visual cortex is more sensitive to a stimulus with figures of different contours and contrast than a flash stimulus, in which the entire retina is stimulated uniformly [1]. Hence, in P-VEP, the visual cortex is stimulated effectively by weak light energy [4]. In human medical science, it has been reported that prolongation of the P100 implicit time was observed in patients with optic neuritis and that remarkable prolongation of the P100 implicit time was observed [3]. Prolongation of the P100 implicit time is suggested to indicate decline of visual recognition. In this study, the P100 implicit time was prolonged in recording P-VEP with eyes corrected to −4, −3, −1, 0, +1 and +2 D, and we thought that a visual recognition declined by a defocus on the retina in these refractive powers. For the above reasons, we concluded that it was necessary to establish the conditions for recording P-VEP in dogs in consideration of the distance to the pattern stimulus display and the refractive power of eyes.

In this study, the N75-P100 amplitude in each dog and the refractive power were not stable. In human medicine, it is 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 [2]. Meanwhile, in a previous report, it was reported that it was difficult to evaluate the N75-P100 amplitude in dogs as a result of large variation [9]. In the present study, the N75-P100 amplitude varied widely at each refractive power, except for −2 D. Given that a consensus concerning evaluation of the amplitude of VEP has not yet been obtained, it is not clear what kind of factor affects the N75-P100 amplitude.

The results of this study suggested that the refractive power of the eye affected the P100 implicit time in P-VEP recording and thus that it was necessary to correct the refractive power strictly in terms of the focus on the retina to record P-VEP. It is suggested that an exact objective visual acuity test in dogs is possible by changing the stimulus pattern size and distance after correcting the refractive power of the eye.

REFERENCES

1. Arieli, A., Sterkin, A., Grinvald, A. and Aertsen, A. 1996. Dynamics of ongoing activity: explanation of the large variability in evoked cortical responses. *Science* **273**: 1868–1871. [Medline] [CrossRef]
2. 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.
3. 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]
4. 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.
5. 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]
6. Itoh, Y., Maehara, S., Okada, K. and Izumisawa, Y. 2010. Pattern-stimulated visual evoked potential in dog: changes in elicited response with pattern size and calculation of visual acu-

ity. *J. Vet. Med. Sci.* **72**: 1449–1453. [Medline] [CrossRef]
7. Jenkins, T. C. and Douthwaite, W. A. 1988. An objective VER assessment of visual acuity compared with subjective measures. *Am. J. Optom. Physiol. Opt.* **65**: 957–961. [Medline] [CrossRef]
8. 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]
9. Mashima, Y., Imamura, Y. and Oguchi, Y. 1997. Dissociation of damage to spatial and luminance channels in early Leber's hereditary optic neuropathy manifested by the visual evoked potential. *Eye (Lond.)* **11**: 707–712. [Medline] [CrossRef]
10. Mizota, A., Asaumi, N., Takasoh, M. and Adachi-Usami, E. 2007. Pattern visual evoked potentials in Japanese patients with multiple sclerosis without history of visual pathway involvement. *Doc. Ophthalmol.* **115**: 105–109. [Medline] [CrossRef]
11. Müller, W. and Schöneich, H. 1989. Relations between visual acuity, refraction and the pattern reversal visual-evoked cortical potential in aphakia. *Ophthalmologica* **198**: 89–94. [Medline] [CrossRef]
12. Nakayama, N., Arakawa, K. and Tarumoto, Y. 1996. Visual function test in beagle with electro-physiological methods (ERG and VEP)—second report—. *Anim. Eye Res.* **15**: 148–154.
13. 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]
14. Saitoh, E., Adachi-Usami, E., Mizota, A. and Fujimoto, N. 2001. Comparison of visual evoked potentials in patients with psychogenic visual disturbance and malingering. *J. Pediatr. Ophthalmol. Strabismus* **38**: 21–26. [Medline]
15. Sokol, S., Hansen, V. C., Moskowitz, A., Greenfield, P. and Towle, V. L. 1983. Evoked potential and preferential looking estimates of visual acuity in pediatric patients. *Ophthalmology* **90**: 552–562. [Medline] [CrossRef]