Evaluation of blink parameters obtained by electrical stimulation in general anaesthetised dogs

Mehmet Nur ÇETİN[1,a,✉], Yusuf Sinan ŞİRİN[1,b]

[1]Mehmet Akif Ersoy University Department of Surgery, Burdur, Türkiye

[a]ORCID: 0000-0003-2610-8477; [b]ORCID: 0000-0003-1322-7290

ARTICLE INFO

Article History
Received : 27.11.2023
Accepted : 05.12.2024
DOI: 10.33988/auvfd.1396530

Keywords
Blink reflex
Dog
Electromyography
Minimal alveolar concentration

✉**Corresponding author**
mncetin@mehmetakif.edu.tr

How to cite this article: Çetin MN, Şirin YS (2025): Evaluation of blink parameters obtained by electrical stimulation in general anaesthetised dogs. Ankara Univ Vet Fak Derg, 72 (2), 231-236. DOI: 10.33988/auvfd.1396530.

ABSTRACT

The aim of this study was to evaluate the electrophysiological relationship between blink parameters R_1, R_2, R_3 and R_C and general anaesthesia in dogs. The study included 16 dogs that were brought for castration or ovariohysterectomy, did not show any cranial neurological signs, no signs of ocular disease, and had not recently used analgesic or sedative drugs. The end tidal minimal alveolar concentration (ETMAC) value was kept constant at 2.9 in dogs that were anaesthetically maintained with sevoflurane for the surgical procedure. After the procedure, supramaximal electrical stimulation was applied to the supraorbital nerve at each 0.1 ETMAC decrease starting from 2.9 ETMAC and blink parameters (R_1, R_2, R_3, R_C) were recorded and evaluated from both orbicularis oculi muscles. In the evaluation, R_1 parameter was obtained at 1.1-1.4 ETMAC values in all cases, R_2 parameter was most commonly obtained at 1.0-1.1 ETMAC values in 14 cases, R_3 parameter was most commonly obtained at 1.1 ETMAC value in 14 cases, R_C parameter was most commonly obtained at 0.9 ETMAC value in 7 cases. As a result, it was revealed at which ETMAC values the blink parameters were obtained under sevoflurane anaesthesia.

Introduction

The blink reflex is an eyelid closure in response to an exteroceptive-nociceptive stimulus (5). The blink reflex is generally considered a trigemino-facial reflex (9). In clinical practice, the blink reflex is usually elicited by mechanical stimulation of the cornea or eyelashes, electrical stimulation of the supraorbital branch of the trigeminal nerve, or touching the glabellar region (16). Recording the electromyographic (EMG) activity from the orbicularis oculi, however, provides quantitative information about the refex circuit. The most commonly used sensory stimulus for eliciting the blink reflex is a brief electrical stimulation applied to the supraorbital nerve (8). Stimulation of the supraorbital nerve (6) elicits one ipsilateral early response (R_1) and two late responses, one ipsilateral (R_2) and one contralateral (R_C) (1). The R_1 is seen only on the side of stimulation and is a simple pontine reflex. The R_2 and R_C are observed on both sides after a unilateral stimulus, and the responses occur synchronously. The R_2 and R_C are relayed through a more complex pathway that involves the pons and lateral medulla oblongata (3). The R_3 response was not noticed until 20 years after the first electrical stimulation study (16). The neuronal synapses of the R_3 response are less well known, but they are believed to be mediated by a polysynaptic neuronal circuit in the medulla oblongata or rostral portions of the cervical spinal cord (15).

General anesthesia provides a reversible loss of consciousness, immobility, muscle relaxation, and loss of sensation in the whole body with the administration of one or more anesthetic agents (17). It provides controlled elimination of sensation by reversibly depressing the central nervous system. Motor responses and sensitivity of animals under general anesthesia to external harmful stimuli are reduced (18). The concept of the minimum alveolar concentration (MAC), which is defined as the volumetric concentration of an inhaled anesthetic that prevents movement in response to a noxious stimulus in

50% of subjects, remains the most used parameter to guide anesthetic depth during inhalational anesthesia (14). In practice, it is a measure of anesthetic effect that determines the level of inhibition of all painful stimuli and muscle movements (2).

Animal and human studies have shown that an electrically evoked blink reflex is suppressed during sedation and anesthesia (12, 13). Therefore, measuring the blink reflex may reflect the depression of reflex arcs induced by anaesthetics (13).

The aim of this study was to evaluate the electrophysiological relationship between blink parameters R_1, R_2, R_3 and R_C and general anesthesia in dogs and to evaluate the relationship between ETMAC and responses affected by the level of consciousness.

Materials and Methods

Animals: This study was conducted with the approval of Burdur Mehmet Akif Ersoy University Experimental Animals Ethics Committee (Decision no: 401). Sixteen dogs of various breeds, ages, sex and body weights brought to Burdur Mehmet Akif Ersoy University Animal Hospital were included in this study. Cranial nerve examinations were conducted, and the animals brought in were found to have no neurologic clinical problems. They also showed no signs of eye disease and reported no use of analgesics or sedatives. These dogs underwent castration or ovariohysterectomy procedures and were classified as ASA (American Society of Anesthesiologists) status I.

Anesthesia: Latency, amplitude, duration and differential latency data of R_1, R_2, R_3 and Rc parameters were collected while the patient was under general anesthesia. For antibiotherapy, cefazolin (20 mg/kg, IV) was administered intravenously to each anesthetized patient. Propofol was administered intravenously for induction until the jaw tone disappeared. The animal was then orotracheally intubated and connected to an anesthesia device (Dräger, Primus, Lübeck, Germany). After connection, the ETMAC value was adjusted to allow the patient to enter a sufficient depth of anesthesia until the end of surgery, and anesthesia was maintained with sevoflurane. A balanced electrolytic solution infusion at a dose of 5 ml/kg/h was started in all patients. Immediately afterwards, meloxicam (0.2 mg/kg, SC) was administered as an analgesic.

Electromyography: Electromyographic stimulations and recordings were performed with a five-channel EMG System (Medelec Synergy, Oxford Instruments, UK) with a total scan time of 100 ms, a sensitivity of 500 mV, and a sampling rate of 10 kHz. The blink reflex test was performed by stimulating the supraorbital nerve with silver needle electrodes (2-2.5 mm). To stimulate the eye, the cathode was placed along the supraorbita of the frontal bone, 1 cm dorsally to the medial canthus of the eye, where the supraorbital nerve exits the orbital space, and the anode was used as a reference electrode. Needle electrodes were placed on the lateral parts of the right and left ventral eyelids to obtain recordings from the orbicularis oculi muscle. The needle electrode placed on the nose was used as a ground (Figure 1).

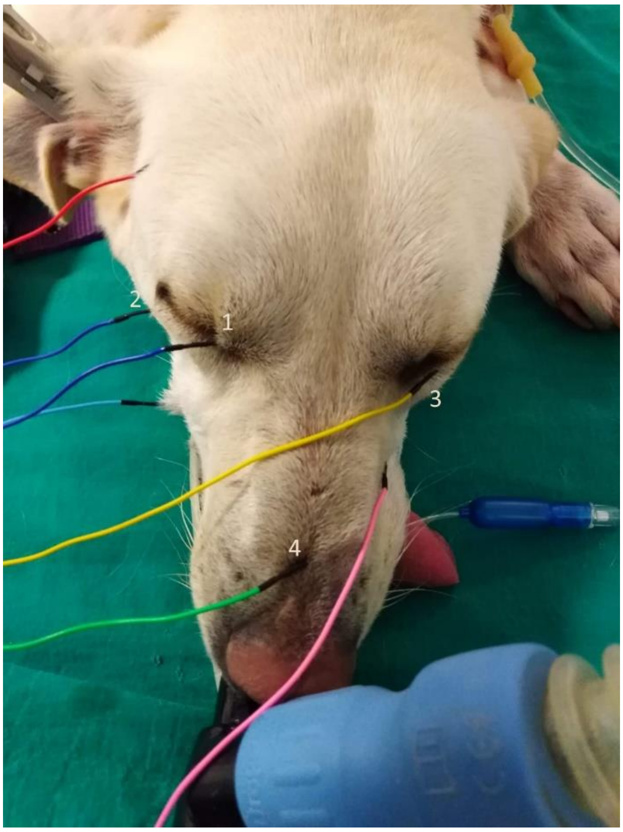

Figure 1. Placement of needle electrodes during electromyography recording includes 1) cathode stimulating electrode, 2) ipsilateral recording electrode, 3) contralateral recording electrode, 4) ground electrode.

After the vaporizer was turned off at the end of the operation, supramaximal stimuli in the form of 0.1 millisecond square waves were given for each 0.1 ETMAC value decrease, starting from 2.9 ETMAC, while the patient was under deep anesthesia. Each stimulus was applied three times, depending on the changing ETMAC value, and the stimuli were administered at 15-second intervals to prevent habituation of the response. In each patient, the stimulus was given only from the right supraorbital nerve and recordings were taken from the right and left orbicularis oculi. Recordings were taken until the R_C value was revealed. In addition, the ETMAC values at which the blink parameters appeared were also recorded.

DOI: 10.33988/auvfd.1396530

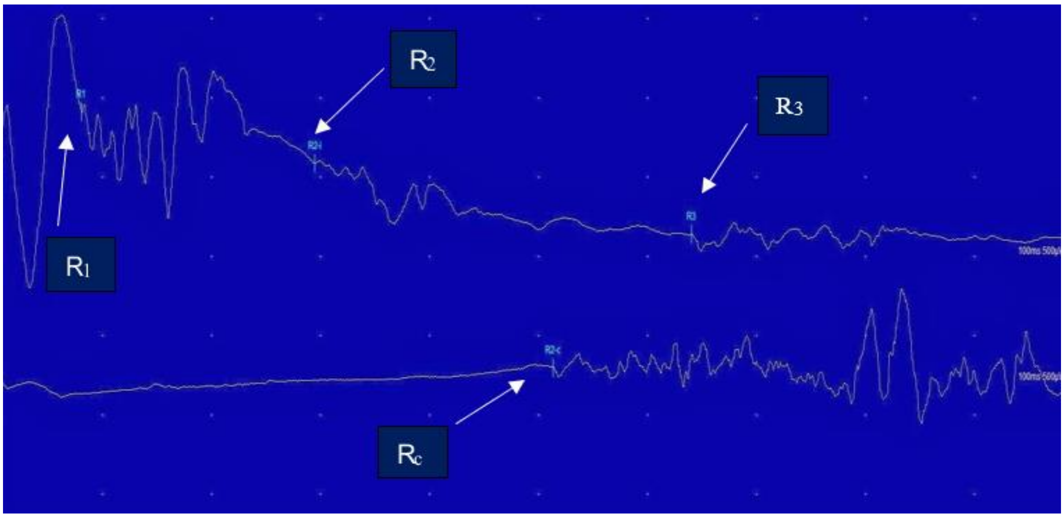

Figure 2. Blink reflex recording of case 9, in which parameters with a MAC value of 0.9 were obtained.

Latency, amplitude, duration and differential latency values of R_1, R_2, R_3, R_C parameters were measured (Figure 2). Latency values were measured from the stimulus artefact to the onset of the reflex components. Amplitude was measured as the distance between parallel lines drawn at the base and the peak of the trace. To measure the duration, the vertical lines drawn at the beginning and end of the tracing were measured. The differential latency value was calculated by taking the difference of the R_2 and R_c values.

Statistical analysis: Descriptive statistics were made on the data, and "Arithmetic Mean + Standart Deviation" for continuous variables and "n, %n" for categorical variables. The correlations between the variables obtained and ETMAC values were analyzed by Spearman's correlation analysis. In all statistical evaluations, values with $p<0.05$ were considered statistically significant. The SPSS 14.01 package programme was used for statistical analysis.

Results

Among cases, all mixed breed, 11 were female (68.80%) and 5 were male (31.30%). The body weights of the cases ranged from 13-22 kg (21.31±1.41), while their ages fell within the 2-3 years range (3.13±0.24).

The latency of the R_1 parameter displayed a polyphasic waveform and demonstrated remarkable stability. In contrast, the latency of the R_2 parameter also exhibited a polyphasic waveform but with greater variability and less stability compared to R_1. The R_C parameter exhibited a polyphasic waveform and appeared after R_2. The R_3 parameter exhibited a polyphasic waveform with a highly variable delay time (Table 1).

The R_1 component exhibited a higher amplitude value compared to the other parameters. The mean amplitude values of the R_2 and R_C components were found to be lower than the mean amplitude value of the R_1 component. The R_3 component had a small amplitude value (Table 2). R_c had the longest duration, R_1 and R_2 times were close to each other (Table 3).

Table 1. Average latency values and differential latency values of parameters R_1, R_2, R_3 and Rc in milliseconds (msec).

Variables (msec)	n	Arithmetic mean	Standard deviation
Latency R_1	303	10.98	0.16
Latency R_2	92	32.68	0.88
Latency Rc	17	48.96	3.12
Latency R_3	71	64.86	0.91
Differential Latency	15	12.56	3.11

n: Number of traces obtained from all cases.

Table 2. Average amplitude values of parameters R_1, R_2, R_3 and R_C in millivolt (mV).

Variables (mV)	n	Arithmetic mean	Standard deviation
Amplitude R_1	303	523.32	24.02
Amplitude R_2	92	197.99	14.87
Amplitude Rc	17	353.79	56.52
Amplitude R_3	71	315.26	22.02

n: Number of traces obtained from all cases.

Table 3. Duration values of parameters R_1, R_2 and Rc in milliseconds (msec).

Variables (msec)	n	Arithmetic mean	Standard deviation
Duration R_1	303	10.40	0.29
Duration R_2	92	10.10	0.52
Duration R_C	17	23.03	4.24

n: Number of traces obtained from all cases.

DOI: 10.33988/auvfd.1396530

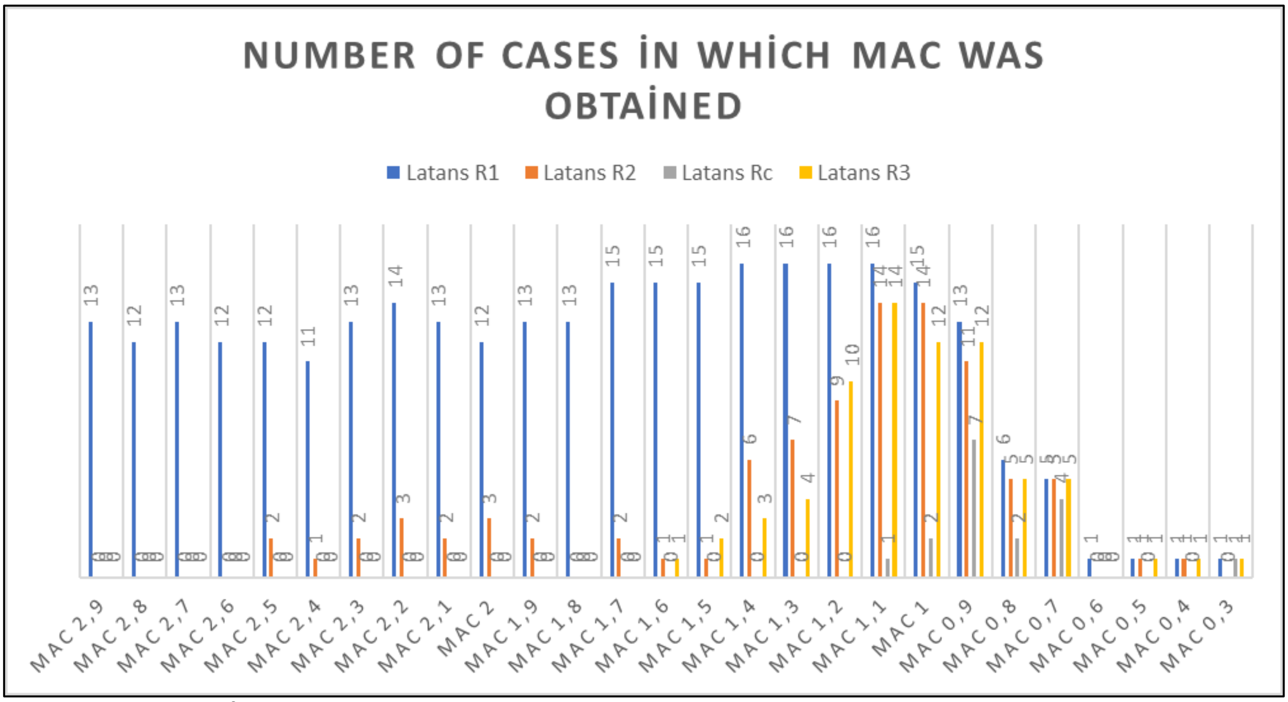

Figure 3. MAC values from which blink parameters are obtained.

The R_1 parameter was obtained in 13 cases at an ETMAC concentration of 2.9, which is accepted as an indicator of deep anesthesia (surgical anesthesia) for dogs, but it was obtained at later ETMAC values in 3 cases. The R_1 parameter was obtained between 1.5 and 1.7 ETMAC in 15 cases (93.80%) and between 1.1 and 1.4 ETMAC in all cases (100%). The R_2 parameter was initially obtained at a ETMAC concentration of 2.5 in only two cases (12.50%). It was most common in 14 cases (87.50%) at 1.0 to 1.1 ETMAC concentrations. The R_3 parameter was initially obtained at a ETMAC concentration of 1.6 in only one case (6.30%). The R_3 parameter was obtained at 1.1 ETMAC concentration in 14 cases (87.50%) at most. The R_C parameter was initially obtained at a ETMAC value of 1.1 in only one case (6.30%). The R_C parameter was obtained in 7 cases (43.80%) at 0.9 ETMAC concentration. The mean ETMAC value for the R_C value was 0.88 (Figure 3).

A significant relationship was found between R_1 latency ($p<0.001$), R_2 latency ($p<0.001$), and ETMAC. No significant relationship was found between R_C latency ($p=0.208$), R_3 latency ($p=0.538$), and ETMAC. A statistically significant relationship was observed between the amplitudes of the R_1 ($p<0.001$), R_2 ($p<0.001$), R_C ($p=0.038$), and R_3 ($p=0.035$) parameters and the ETMAC. A statistically significant relationship was observed between the durations of the R_1 ($p<0.001$) and R_2 ($p<0.001$) parameters and ETMAC. There was a statistically insignificant relationship between the durations of the R_C ($p=0.329$) parameters and ETMAC.

Discussion and Conclusion

R_1 is stable and reproducible, usually in a two- or three-phase form. On the other hand, R_2 has a polyphasic shape; it tends to change and become habitual after repeated stimulation (19). Late reflexes are observed on both sides (ipsialteral R_2 and contralateral R_C) after unilateral stimulation and occur synchronously, with a delay of R_C delay slightly longer than R_2. R_3 has also been found to have a highly variable polyphasic muscle potential but a longer latency than R_2 (3). In present study, the R1, R2, RC and R3 parameters were found to be equivalent to previous human studies and the dog study of Anor et al. (3).

The amplitude of R_1 and R_2 is metrically low due to variability and signal noise (4). Other researchers have reported that amplitude and duration have no value due to the large standard deviation and large variability (19). In the study, the R_1 component had a larger amplitude value than the other parameters. The R_3 component had a small amplitude value. The mean amplitude values of the R_2 and R_C components were found to be lower than the mean amplitude value of the R_1 component. Amplitude values were found to be close to the study of Anor et al. (3) in beagle dogs, but it did not indicate an intersubject value since it had a high standard deviation.

In a study, it was revealed that the most resistant component to propofol was R_1, the first component of the blink reflex, R_2, the second component of the blink reflex, was suppressed more than R_1, and R_3 was deeply suppressed. It was found that the R_3 component was more sensitive than the R_2 component and the most resistant

DOI: 10.33988/auvfd.1396530

component was R_1 (13). Late responses (R_2 and R_C) are highly affected by the level of consciousness, and R_C disappears under general anaesthesia due to suppression of the polysynaptic reflex pathway to the contralateral facial motor nucleus (3). In the study, it was observed that blink parameters were suppressed during anesthesia. Anesthetic agents suppress reflex pathways by acting on GABA and glycine receptors, which is thought to be the cause of the suppression of the parameters with the depth of anesthesia. Obtaining the R_1 parameter at a concentration of 2.9 MAC in 13 cases showed that R_1 was the most resistant parameter to anesthesia. The R_2 parameter obtained at a concentration of 2.5 MAC showed that R_2 was less resistant than R_1, but more resistant than R_3, which was initially obtained at a concentration of 1.6 MAC. The least resistant parameter was recorded as the R_C parameter, which occurs when the animal regains consciousness. At the same time, the MAC value at which the R_C value was obtained was 0.88, which can be considered the average MAC value for dogs at which the effect of general anesthesia disappears. The dogs could not tolerate any other stimulus once the R_C value appeared. The R_2 value was most commonly obtained between 1.1 and 1.0 MAC, but the dogs were not responding to any stimulus when R_2 was obtained. But since prior research has shown that consciousness level influences R_2 values, it was assumed that surgical anesthesia might have vanished in the MAC ranges where this value was measured.

Marelli and Hillel (10), stated that no parameters would appear during surgical anaesthesia in patients anesthetized with isoflurane and halothane inhalation anaesthetics. At the same time, Moller and Jannetta (11) stated that blink reflex parameters cannot be revealed during surgical anaesthesia using modern inhalation anesthetics in humans. Once a short train of stimuli was used, it became the standard for eliciting motor evoked potentials (MEPs) under general anesthesia. The discovery that a short train of stimuli can elicit an R_1 component of the blink reflex in a patient under general anesthesia, when a single stimulus cannot in most patients, is very similar to the history of MEPs. The efficacy of a short train of stimuli to overcome the inhibitory action of anesthetics is very likely due to temporal summation and building-up of excitatory postsynaptic potentials in the interneuronal chain involved in the blink reflex in the brainstem (7). In the study obtained, the R_1 value at the end tidal MAC concentration was 2.9, which is accepted as an indicator of deep anaesthesia (surgical anaesthesia) for dogs, in 13 cases, and was obtained later in 3 cases. This suggests that the R_1 parameter may occur during surgical anesthesia in dogs anesthetized with sevoflurane. The values of R_2, R_C, and R_3 could not be obtained at first, but were obtained later. This suggests that blink parameters can be obtained during sevoflurane anesthesia with a short train stimulus.

As a result, the mean latency, amplitude, duration and differential latency values of the blink parameters R_1, R_2, R_3 and R_C obtained under sevoflurane anesthesia were presented in the study. At the same time, the suppressive effect of anesthesia on the blink reflex, at which MAC values it disappears and at which MAC value which blink parameter is obtained were revealed. The study was conducted on mixed breeds and it is thought that species-specific studies can also be conducted.

Acknowledgements

This study was derived from the master's thesis of the first author.

Financial Support

This research received no grant from any funding agency/sector.

Ethical Statement

This study was conducted with the approval of Burdur Mehmet Akif Ersoy University Experimental Animals Ethics Committee (Decision no: 401).

Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

MNÇ and YSŞ conceived and planned the experiments. MNÇ and YSŞ carried out the experiments. MNÇ and YSŞ contributed to the interpretation of the results. MNÇ took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

References

1. **Aktekin B, Yaltkaya K, Ozkaynak S, et al** (2001): *Recovery cycle of the blink reflex and exteroceptive suppression of temporalis muscle activity in migraine and tension-type headache.* Headache, **41,** 142-149.
2. **Alvillar BM, Boscan P, Mama KR, et al** (2012): *Effect of epidural and intravenous use of the neurokinin-1 (NK-1) receptor antagonist maropitant on the sevoflurane minimum alveolar concentration (MAC) in dogs.* Vet Anaesth Analg, **39,** 201–205.

3. **Anor S, Espadaler JM, Pastor J, et al** (2000): *Electrically induced blink reflex and facial motor nerve stimulation in beagles.* J Vet Intern Med, **14,** 418– 423.
4. **Aramideh M, Ongerboer de Visser BW** (2002). *Brainstem reflexes: Electrodiagnostic techniques, physiology, normative data, and clinical applications.* Muscle Nerve, **26,** 14-30.
5. **Bernard JM, Pereon Y** (2005): *Nerve stimulation for regional Anesthesia of the Face: Use of the Blink Reflex to Confirm the Localization of the Trigeminal Nerve*. Anesth Analg, **101,** 589 –91.
6. **Cruccu G, Agostıno R, Berardellı A, et al** (1986): *Excitability of the corneal reflex in man.* Neurosci Lett, **63,** 320-324.
7. **Deletis V, Urriza J, Ulkatan S, et al** (2009): *The feasibility of recording blink reflexes under general anesthesia.* Muscle Nerve, **39(5),** 642–646.
8. **Ferreira A, Vide S, Felgueiras J, et al** (2020): *Electromyographic assessment of blink refex throughout the transition from responsiveness to unresponsiveness during induction with propofol and remifentanil.* J Clin Monit Comput, **35(6),** 1279-1289.
9. **Giffin NJ, Kowacs F, Libri V, et al** (2003): *Effect of the adenosine A1 receptor agonist GR79236 on trigeminal nociception with blink reflex recordings in healthy human subjects.* Cephalalgia, **23,** 287–292.
10. **Marelli RA, Hillel AD** (1989): *Effects of general anesthesıa on the human blink reflex.* Head Neck, **11,** 137-149.
11. **Moller AR, Jannetta PJ** (1986): *Blink reflex in patients with hemifacial spasm. Observations during microvascular decompression operations.* J Neurol Sci, **72,** 171.
12. **Mourısse J, Gerrıts W, Lerou J, et al** (2003): *Electromyographic assessment of blink and corneal reflexes during midazolam administration: useful methods for assessing depth of anesthesia.* Acta Anaesth Scand, **47,** 593-600.
13. **Mourısse J, Lerou J, Zwarts M, et al** (2004): *Electromyographic assessment of blink reflexes correlates with a clinical scale of depth of sedation/anaesthesia and BIS during propofol administration.* Acta Anaesth Scand, **48,** 1174-1179.
14. **Müller J, Plöchl W, Mühlbacher P, et al** (2022): *The Effect of Pregabalin on the Minimum Alveolar Concentration of Sevoflurane: A Randomized, Placebo-Controlled, Double-Blind Clinical Trial.* Front Med, **9,** 883181.
15. **Romaniello A, Valls-Sole J, Iannetti GD et al** (2001): *Nociceptive Quality of the Laser-Evoked Blink Reflex in Humans.* J Neurophysiol, **87,** 1386-1394.
16. **Smit AE** (2009): Blinking and the Brain: Pathways and Pathology. Avaliable at http://hdl.handle.net/1765/14477. (Accessed Feb 15, 2019).
17. **Thomas JA, Lerche P** (2017): Introduction to Anesthesia. 1-6. In: JA Thomas, P Lerche (Eds), Anesthesia and Analgesia for Analgesia for Veterinary Technicians. 5th ed. Elsevier, St. Louis, Missouri.
18. **Tranquilli WJ, Grimm KA** (2015): Introduction: Use, Definitions, History, Concepts, Classification, and Considerations for Anesthesia and Analgesia. 3-10. In: KA Grimm, LA Lamont, WJ Tranquilli, SA Greene, SA Robertson (Eds). Lumb and Jones Veterinary Anesthesia and Analgesia. 5th ed. John Wiley & Sons, Iowa, USA.
19. **Yoo J, Cho J, Kim D** (2012): *Utilization of averaging process of blink reflex to ımprove diagnosis of facial nerve palsy.* J Exp Biomed Sci, **18,** 391-398.

DOI: 10.33988/auvfd.1396530