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Comparison of the Effects of Isoflurane and Sevoflurane on BAER and Tympanometry in Dogo Argentino Breed Puppies

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Abstract: Isoflurane and sevoflurane are the most frequently used volatile anesthetic agents in veterinary practice yet their effects on the brain are not fully understood. In this study, it was aimed to compare the brainstem auditory evoked response (BAER) test data recorded during stable general anesthesia periods in specific MAC (Minimal Alveolar Concentration) values of sevoflurane (1.7%) and isoflurane (2.1%). The patients who were evaluated as healthy according to physical, video-otoscopy, and tympanometry examination results were included in the study. Thus, detailed breed specific reference tympanometric values were obtained in a certain age range. In the study, 12 (9 male and 3 female) *Dogo Argentino* breed, 8-12 weeks old, healthy dogs were used, which were maintained with anesthesia with isoflurane (n: 6) and sevoflurane (n: 6). As well as tympanometry data, the latency, amplitude, V/I amplitude ratio, and interpeak latencies between the two groups BAER records were compared. Data were analyzed using Student's t-test. Statistical significance was determined as P<0.05. The study design is prospective, clinical, blind, cross-study. In conclusion, the brain appears to be more suppressed for the minimal alveolar concentration values determined in this study in the isoflurane group, but tympanometry values were not affected.

Keywords: Anesthesia, BAER, Dogo argentino, MAC, Tympanometry.

Dogo Argentino Irkı Yavru Köpeklerde Izofluran ve Sevofluran'ın BAER ve Timpanometre Üzerindeki Etkilerinin Karşılaştırılması

Öz: İzofluran ve sevofluran, veteriner pratiğinde en sık kullanılan uçucu anestezik ajanlardır ancak beyin üzerindeki etkileri tam olarak anlaşılamamıştır. Bu çalışmada, stabil genel anestezi dönemlerinde kaydedilen beyin sapı işitsel uyarılmış yanıt (BAER) testi verilerinin sevofluran (%1.7) ve izofluranın (%2.1) spesifik MAK (Minimal Alveolar Konsantrasyon) değerlerinde karşılaştırılması amaçlanmıştır. Fiziki, video-otoskopik ve timpanometrik muayene sonuçlarına göre sağlıklı olarak değerlendirilen hastalar çalışmaya dahil edildi. Böylece detaylı ırka özgü referans timpanometrik değerler belirli yaş aralığında elde edildi. Çalışmada izofluran (n: 6) ve sevofluran (n: 6) ile anestezisi idame ettirilen 8-12 haftalık, 12 (9 erkek ve 3 dişi) Dogo Argentino ırkı köpek kullanıldı. Timpanometri verilerinin yanı sıra, BAER kayıtları iki grup arasındaki latans, amplitüd, V/I amplitüd oranı ve interpik latansları karşılaştırıldı. Veriler Student's t testi kullanılarak incelendi. İstatistiksel anlamlılık P<0.05 olarak belirlendi. Çalışma tasarımı prospektif, klinik, kör, çapraz çalışmadır. Sonuç olarak, izofluran grubunda bu çalışmada belirlenen MAK değerleri için beyin daha baskılanmış görünmektedir ancak timpanometri değerleri etkilenmemiştir.

Anahtar Kelimeler: Anestezi, BAER, Dogo argentino, MAK, Timpanometri.

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INTRODUCTION

Volatile general anesthetics are used commonly, yet their mechanisms of action are complex and remain obscure (1,2). Although isoflurane and sevoflurane belong to the same class of volatile general anesthetics, their effects upon single cortical units and local field potentials were quite different according to a study on rabbits. The conclusion of a recent study is that their effects on anesthesia are dependent mainly on the changes in GABA/glutamate neurotransmission induced by each drug (1). γ-Aminobutyric acid-A receptors are also widely expressed in the central and peripheral nervous systems and mediate fast postsynaptic inhibition. In dogs, the distribution of $GABA_A$ receptor subunits only in nodose ganglion has been investigated (3). Furthermore, MAC remains a clinically useful measure of the potency of inhaled anesthetics (4).

It is especially important to screen the breeds in which congenital deafness is common in order to evaluate hearing in dogs (5). *Dogo Argentino* dogs are counted as one of 90 breeds genetically prone to unilateral or bilateral congenital deafness (6).

Video-otoscopy has many advantages for the assessment, diagnosis, and treatment of otitis. To evaluate the tympanic cavity, it is ideal to combine otoscopy with computed tomography or magnetic resonance imaging. So the clinician knows if the tympanic cavity should be sampled or examined, which decreases the risk of introducing bacteria and/or hemorrhage, which could predispose to otitis media in cases with a normal middle ear (7).

Tympanometry is an important component of the basic audiological examination. An objective examination of the middle ear can best be done with tympanometry (8). The purpose of tympanometry is to evaluate hearing objectively in light of the integrity and compliance of the eardrum, mobility of the ossicular chain, the function of the middle ear muscles and connections, and the size of the outer ear canal (9).

Waves obtained within the first 10 ms following stimulation are considered as early latency components and represent BAER (10). The BAER test is the only objective diagnostic method that can provide a precise diagnosis of deafness widely in the assessment of the hearing function of animals and humans (6). In veterinary clinical practice, the BAER test is mainly used as a screening mechanism; however, it has great potential as a diagnostic tool for central auditory dysfunction (5).

The aim of this study was to obtain breed-specific BAER and tympanometry values from Dogo Argentino breed puppies at a certain age range and anesthesia depth.

MATERIALS and METHODS

In our country, a hearing test certificate is requested from puppies of some breeds bred under federations. Twelve, 8-12 week old healthy *Dogo Argentino* breed pups, which were brought to Mehmet Akif Ersoy University Animal Hospital Surgery Clinic with this request, created the study material. The presented study was carried out with the decision of Mehmet Akif Ersoy University's Animal Experiments Local Ethics Committee with the number 82/681.

After physical examinations of all cases (n:12), video-otoscopic examination of both ears was performed using Karl Storz tele pack Vet X® and tympanometric examination with Interacoustics AT235h® tympanometer using a 226-Hz probe; images and tympanograms were recorded.

After the video-otoscopic examination, the ears were evaluated individually by using 13mm mushroom tips in the tympanometric examinations of the patients who were taken to general anesthesia. Ear volume, compliance, pressure, gradient values and acoustic reflex data were recorded. Afterward, BAER tests were performed with Synergy CareFusion 5-channel EMG-EP® device after the patient was placed in the ventral recumbency and ear plugs and subdermal needle electrodes were placed.

In all these examinations, dogs found to be completely normal were included in the study. Dogs with unilateral or bilateral sensorineural deafness (diagnosed with the absence of the BAER waveform) and those less than 8 weeks or over 12 weeks old were excluded from the study.

After the induction of anesthesia was achieved with a mask, the cases were intubated with an appropriately sized cuffed endotracheal tube and connected to an anesthesia device (Dräger, Primus® model, Germany) operating with a semi-open/closed-circuit system. Anesthesia was maintained by maintaining end-tidal concentrations of isoflurane (Isoflurane®, 100mL Abbott) to one of the two groups and the end-tidal concentrations of sevoflurane (Sevorane®100mL Abbott) to 1.7% and 2.1%, respectively. Thus, the cases could be maintained with spontaneous ventilation throughout the procedure.

During general anesthesia, URIT A63A® (URIT Medical Electronic, China) bedside monitor and anesthesia device utilized to record capnograph, pulse oximeter, pulse rate, respiratory rate, core body temperature (with esophageal temperature probe), ECG, tidal volume, minute volume, peak inspiratory pressure, plateau pressure, non-invasive blood pressure values. End-tidal CO_2 value was provided to remain in the range of 40-45 mmHg during anesthesia.

Since the BAER records were affected by the body temperature changes of the patient, the central body temperatures of the patients were monitored, and the heated table and infrared lamp were used to prevent hypothermia during anesthesia. The mean arterial pressure was measured noninvasively at 10-minute intervals and remained within the normal range throughout the procedure. The stimulation intensity was set at 70dB NHL (normal hearing level). Headphones with the same type of foam tip were used for conduction of the sound stimulus. Teflon-coated stainless steel needles were placed subcutaneously into the vertex and the mastoid region of the ipsilateral ear. The needle with the same properties was placed in the neck area as the ground electrode. It is provided that the impedance of the electrodes is below 5kΩ. The latency, amplitude, V/I amplitude ratio, and I-III, I-V, III-V interpeak latencies of the obtained peaks (I to VI) were evaluated together with their differences with the ipsi and contralateral ear. The resulting latency, amplitude, and central conduction velocity were used to determine the fit in the traces between the two ears. All dogs recovered without an anesthetic problem.

Statistical Analyses

A total of 12 (9 male and 3 female) *Dogo Argentino* breeds were included in the study at the age of 8-12 weeks. Student t-test was used for BAER and tympanometric results. During video-otoscopy, bright, striae, semi-transparent pars tensa and opaque, vascular pars flaxida were observed. The images obtained were recorded. During tympanometry, ear volume, pressure, compliance, gradient data and ipsi and contralateral acoustic reflex tests were also performed. The results obtained from tympanograms are given in Table 1. Acoustic reflex could not be obtained clearly in any of the cases. Right and left ears of the patients were evaluated separately for tympanometric results.

Peak latencies and amplitudes of the waves (I to VI) and I-III, III-V and I-V interpeak latencies and V/I amplitude ratios were measured and compared between the groups by Student's t-test. Study data were evaluated using means and standard errors (mean± standard deviation). After all the data were collected, BAER latencies and amplitudes, which appeared with the use of two different anesthetics, were compared. Values are as in Table 2. The results obtained from the right and left ears of each case were averaged for statistical analysis.

RESULTS and DISCUSSION

Congenital deafness in the domestic dog is usually related to the presence of White pigmentation (11,12). The *Dogo Argentino* breed has a common white body phenotype, and lesions are classified as cochleo-vascular (13). In a study that included 243 dogs of four breeds with a predisposition to congenital deafness, the prevalence was determined as 37.5% (14). Therefore, it is important to carry out a comprehensive ear examination and BAER test in the early period belonging to the *Dogo Argentino* breed, which has a high incidence of deafness and is the material of this study.

Anamnesis, clinical examination, impedance audiometry, and BAER tests are used for the diagnosis of hearing loss (15). Video-otoscopy is a practical and effective tool for the diagnosis and management of otitis externa and media (16). The tympanic membrane has a thin, taut, bright pars tensa and a loose, opaque, pink, triangular pars flaccida region (17). Normal vascularization of it to the manubrium and membrane should be clearly visible. Some changes that can be seen in the tympanic membrane include thickening, edema, erythema, and the appearance of fluid behind the membrane (18). With video-otoscopy, the structure of the outer ear canal and the tympanic membrane can be displayed in detail in a practical way. No otitis externa and/or media-related changes were detected in any of the patients in the study. In addition, the color and hair structure unique to the *Dogo Argentino* breed drew attention (Figure 1).

Figure 1. Normal video-otoscopic views of case 6.
Şekil 1. Olgu 6'nın normal video-otoskopik görünümü.

In a study performed by Strain et al. (19) in 16 awake adult dogs, the ear canal volume was reported as 1.96 ml, compliance 0.53 ml, and peak pressure 27.8ml. The lower mean ear volume was found in this study (1.19±0.23 ml) might depend on the younger age of the cases. Apart from this, the different tympanometry devices used in the study and the sealing location of the tip can be some of the reasons for the difference in the results. Compared to this article, compliance was measured at a close value (0.42 ml±0.17). In a study conducted in humans (20), it was emphasized that compliance decreases only with aging in the right ears of older women, and there is no significant difference in compliance, ear volume, and pressure with age. Some researchers have suggested that isoflurane anesthesia causes a higher compliance peak (19,20). However, there was no significant difference between isoflurane and sevoflurane groups for compliance in this study. A mean middle ear pressure [27.58±53.19daPa (decapascal)] very close to the value revealed in the study of Strain et al. (19) was obtained from the cases used in this study and evaluated as normal. The tympanograms of the cases in this study were recorded in order to identify the pathologies related to the middle ear that can be overlooked and to reveal the breed specific values. The fact that it can be obtained from all cases and the consistency of the data obtained has shown that it can be used safely in healthy ears (Figure 2, 3) (Table 1).

Figure 2. Interacoustics AT235h® tympanometer.
Şekil 2. Interacoustic AT235h® timpanometre.

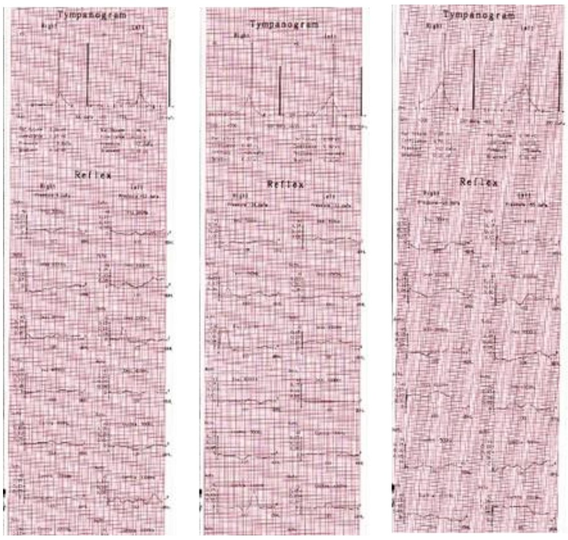

Figure 3. Tympanograms of cases 1, 5 and 9 from left to the right respectively.
Şekil 3. Soldan sağa sırasıyla 1, 5 ve 9. olguların timpanogramları.

Table 1. Comparison of tympanometry values between isoflurane and sevoflurane groups.
Tablo 1. İzofluran ve sevofluran gruplarının timpanometri değerlerinin karşılaştırılması.

Parameter	Direction	Isoflurane	Sevofluran	P	Mean±SD
Ear volume (ml)	right	1.23±0.06	1.30±0.17	-	1.19±0.23
	left	1.09±0.03	1.15±0.06	-	
Compliance (ml)	right	0.46±0.07	0.35±0.03	-	0.42±0.17
	left	0.41±0.06	0.46±0.10	-	
Pressure (daPa)	right	12.7±20	25.5±28	-	27.58±53.19
	left	29.0±16	43.2±26	-	
Gradient (ml)	right	0.20±0.05	0.16±0.02	-	0.18±0.10
	left	0.20±0.07	0.17±0.03	-	

* P<0.05 and ** P<0.001 were considered statistically significant. ml: milliliter. daPa: decapascal.
- : No statistically significant.

The gradient value in humans varies between 0.0-1.0 ml. Gradient value less than 0.2 ml is considered unusually low and is associated with the presence of fluid in the middle ear in human infants (21). In a study comparing healthy children and those with otitis media, the normal gradient was calculated in the range of 0.13-0.97 ml (mean 0.34 ml) for the left ear and 0.07-0.78 ml (mean 0.31 ml) for the right ear. In children with otitis media, the gradient value was determined in the range of 0.01-0.92ml (mean 0.12 ml) for the left ear and in the range of 0.01-0.82ml (mean 0.82 ml) for the right ear (22). The gradient values of 24 ears obtained in this study were measured in the range of 0.08-0.25 ml and an average of 0.18±0.10 ml. Since ear volume, compliance, and pressure values are compatible with those obtained from other dog studies, and other examinations were all normal, it was thought that the low gradient values than human data were depending on the difference of species, breed, and age factors.

In addition to determining the condition of the middle ear, the acoustic reflex can be used to identify the side of the lesion in the auditory pathway, to distinguish between cochlear and retrocochlear pathologies. It also helps to determine the degree of hearing loss and rule out non-organic hearing loss (23). Healthy acoustic reflex data were successfully obtained in a study using a 226-Hz probe and examining dogs aged 5-127 months (24). Adult acoustic reflexes can be obtained in human infants when high probe frequency is used. From the age of six months, the 226-Hz probe frequency can be used in acoustic reflex measurement in tympanometry. There are no normative data in humans using the 226-Hz probe frequency from neonatal period to 6 months of age (23). Failure of obtaining acoustic reflex from the cases included in the study was attributed to the age of 8-12 weeks and the use of the 226-Hz probe. According to the author's knowledge, there is no study about how early in life the acoustic reflex could be obtained with a 226-Hz probe in dogs.

BAER is a test used in the objective examination of hearing. This test shows the brain activities induced by auditory stimulus and reflects the electrophysiological process of the sensory cells from all auditory pathways. After being found in the late 1970s, BAER took its place in the quantitative hearing analysis as a reliable, objective, a reproducible and noninvasive test of hearing functions in dogs. The purpose of the BAER examination is to determine whether deafness (unilateral or bilateral), the severity, and localization of the lesion (central/peripheral hearing pathways). It is also used in conditions such as demyelination, brainstem tumors, vascular insufficiencies, and brainstem injury due to trauma (13,22). The results of a study also revealed the BAER changes in children with otitis media (25). It has been reported that adult BAER waveforms can be obtained in 6-8 week-old dogs (26). Some researchers have emphasized that race is an important factor in BAER measurements (25). For this reason, this study aimed to obtain normal data close to adulthood, which is generally needed, by adding *Dogo Argentino* breed dogs, which are known to have a high incidence of deafness at the age of 8-12 weeks, and avoided breed variations.

It is known that some factors such as anesthetic agents (e.g., sevoflurane, isoflurane, enflurane) and body temperature are associated with increased neural conduction time during anesthesia. An increase in central transmission time causes an increase in III, V, I-III, III-V, and I-V waves and interpeak latencies, except for the first wave (27). Isoflurane is a volatile gas anesthetic commonly used in auditory evoked potential tests in humans and animals for research (28). Because volatile anesthetics suppress brainstem neuronal activity, they cause minor increases in BAER latencies. It is suggested that volatile anesthetics slow the transmission in synaptic transmission. For this reason, the researchers stated that they are less affected on oligosynaptic roads (such as BAER) than polysynaptic roads. In some studies in humans, it was found that preanesthetic BAER latencies obtained from patients increased under general anesthesia (26,27). For these reasons, body temperature and depth of anesthesia (according to end-tidal isoflurane and sevoflurane concentrations) were closely monitored and kept in a certain range in the evaluation of BAER to be able to determine changes.

In veterinary clinical practice, the BAER test is mainly used as a screening mechanism; however, it has great potential as a diagnostic tool in determining central auditory dysfunction. In dogs, the BAER test is commonly used to document whether puppies have congenital hereditary deafness. Evaluation of BAER in the dog depends on comparing the results with appropriately matched normative data (5). In this study, variables were kept as minimum as possible in order to obtain the breed-specific normative data in the most sensitive way and to be a reference in distinguishing them from slightly different or abnormal conditions.

Anesthesia can cause vasodilation and body temperature to drop, causing BAER interpeak intervals to increase (27). In order to avoid changes due to body temperature, the body and the covers were warmed up before the patient was taken to anesthesia, and central body temperature was monitored with the esophageal temperature probe during anesthesia. Since all of the cases included in the study were evaluated only under general anesthesia, the differences in awake and under anesthesia could not be revealed. Although the I-V interpeak latency difference is statistically high only in the isoflorane group in the right ear, the difference between them, like other interpeak latencies, is clinically insignificant. Average right and left wave latencies recorded from cases; II. wave latencies in both sides, left ear IV. wave latency and right ear V. wave latency were statistically significantly higher in the isoflurane group. Except left ear III. wave latency, all other wave latencies were insignificantly higher in the isoflurane group. However, these differences were evaluated as not clinically important.

To assess brainstem integrity, I-III, III-V, and I-V interpeak latencies are evaluated. These should be in the reference range, and there should be no more than a 0.1 ms difference between right and left. More specifically, these values greater than 0.2 ms are considered abnormal, and between 0.1-0.2 ms are considered suspicious in terms of the brainstem (29). In this study, the mean of I-III, III-V, and I-V interpeak latencies between the right and left records was calculated as 0.09, 0.11, and 0.11 ms, respectively. Since these values are the cumulative average of individually healthy records, these results, which are slightly outside the normal limits, were found to be normal. The I-V interpeak latency was found to be statistically significantly higher in the isoflurane group compared to the sevoflurane group in the records taken from the right side and was considered also normal because of no significant difference in other interpeak latencies (Table 2).

Table 2. Comparison of amplitude, latency, and interpeak latency values between isoflurane and sevoflurane groups.
Tablo 2. İzofluran ve sevofluran grupları arasında amplitüd, latans ve interpik latans değerlerinin karşılaştırılması.

	Amplitude (μV)				Latencies (ms)		
Wave	Direction	Isoflurane	Sevoflurane	P	Isoflurane	Sevoflurane	P
I	right	2.68±0.30	2.57±0.18	-	1.90±0.01	1.89±0.02	-
	left	2.70±0.14	2.70±0.14	-	1.93±0.01	1.92±0.02	-
II	right	3.46±0.43	3.37±0.29	-	2.91±0.01	2.82±0.03	*
	left	3.44±0.31	3.60±0.50	-	2.92±0.03	2.83±0.03	*
III	right	1.99±0.51	1.54±0.33	-	3.78±0.08	3.70±0.06	-
	left	1.43±0.28	2.10±0.38	-	3.72±0.06	3.82±0.07	-
IV	right	0.45±0.06	0.70±0.14	-	4.08±0.02	3.98±0.07	-
	left	0.72±0.12	0.78±0.12	-	4.20±0.06	3.95±0.01	*
V	right	1.16±0.22	1.68±0.38	-	4.73±0.02	4.61±0.03	*
	left	1.68±0.40	1.94±0.22	-	4.61±0.04	4.57±0.04	-
VI	right	could not be evaluated			could not be evaluated		
	left						
V/I	Both	0.612±0.28	0.853±0.33	-			
Wave	Direction	Latencies (ms) Interpeak Isoflurane			Sevoflurane		P
I-V	right	2.82±0.05			2.72±0.05		*
	left	2.68±0.04			2.65±0.03		-
I-III	right	1.71±0.12			1.81±0.06		-
	left	1.80±0.07			1.90±0.07		-
III-V	right	0.95±0.09			0.92±0.06		-
	left	0.88±0.08			0.76±0.09		-

* P<0.05 and ** P<0.001 were considered statistically significant. μV : millivolt. ms : millisecond.
- : No statistically significant.

Due to the morphology of BAER waves obtained from dogs, III. and IV. waves cannot always be obtained separately (29). These intertwined waves can cause the latency values to be measured differently. Probably, for this reason, the average latency difference between the right and left ears in groups was found clinically significant only for the IV. wave. Therefore, only the IV. wave was found above the reference limit of 0.2 ms (0.25 ms) (Figure 4, 5).

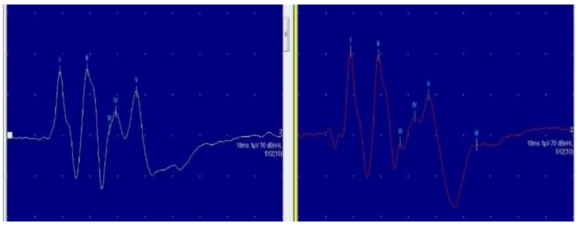

Figure 4. Comparison of normal BAER values obtained from the left and right ear.
Şekil 4. Sol ve sağ kulaktan elde edilen normal BAER değerlerinin karşılaştırılması.

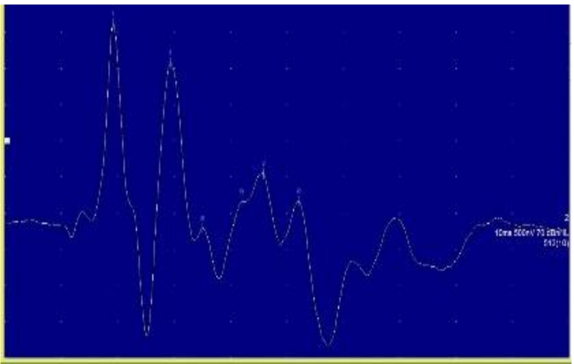

Figure 5. A normal BAER test result was obtained by a "click" stimulus at 70dB NHL level.
Şekil 5. 70dB NHL seviyesinde "klik" uyaranla elde edilen normal bir BAER testi sonucu.

This study shows that the effect of isoflurane and sevoflurane on the results of BAER tests at certain end-tidal values in dogs is not clinically significant. However, the absence of statistically different tympanometric measurements used in the examination of the middle ear was interpreted as it provides sensitive, consistent results in healthy ears and may be useful in clinical practice. Before performing and interpreting the BAERs, physical, video-otoscopic, and tympanometric examinations are recommended by clinicians experienced in this field because many factors needed to be considered when interpreting the BAER test in detail.

Dogs away from hearing loss are necessary for security and military work. For this reason, BAER becoming a part of the selection, health, and performance evaluations will be beneficial in reducing the incidence of hereditary deafness and preventing loss of growth.

As a result, according to author's knowledge, although isoflurane and sevoflurane are the most frequently used volatile anesthetic agents their effects on BAER in dogs were not compared before. Considering the differences in latency and interpeak latency between groups, it can be said that the brain is more suppressed for the minimal alveolar concentration values determined in this study in the isoflurane group compared to both statistically significant and statistically insignificant values. Again, although not statistically significant, it is consistent with the lower V/I amplitude ratio in the isoflurane group. In addition, tympanometry, which can provide very meaningful information in veterinary practice, has not been studied sufficiently. In conclusion, it is important to compare the effects of different anesthetics and gaining a better understanding of their mechanisms of action with breed-specific values. These values can serve as a reference as well as a basis for identifying abnormal conditions and predicting the course of the disease.

Conflict of interest

The authors declare that they have no conflict of interest.

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