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The Effect of Head Size on Bone Conduction Brainstem Auditory Evoked Response in Canines

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THE EFFECT OF HEAD SIZE ON BONE CONDUCTION BRAINSTEM AUDITORY EVOKED RESPONSE IN CANINES

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has been approved as meeting the requirement for the Degree of Doctor of Audiology in the College of Natural and Health Sciences, Department of Audiology and Speech-Language Sciences.

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ABSTRACT

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The Brainstem Auditory Evoked Response (BAER) is the gold standard for testing the auditory system in many animals, including canines. The procedure involves measuring electrical responses that occur at various locations along the auditory pathway and brainstem. Electrical activity occurs as a result of auditory stimulation, presented either via air conduction or bone conduction, and can be measured via small subdermal electrodes. Since this method measures a physiological response to sound, a behavioral response from the animal is not required, resulting in an objective assessment of that animal's auditory function.

Previous studies have been conducted, namely Kemper et al. (2013), in which the effect of head size on the air-conducted BAER in dogs was examined. It was found that there was no significant difference on the response waveform between various head sizes. Munro, Paul, et al. (1997) conducted a study to establish normative data for bone conduction BAER waveforms in dogs. They reported a consistent observable difference in Wave latency between the two breeds tested, one small breed and one large breed. The purpose of the following study was to further investigate how head size affects the waveform of a bone conduction BAER in dogs, following the findings of Munro, Paul, et al. (1997) and Kemper et al. (2013). The following research questions were investigated: What effect does head size have on the absolute latency of Wave V for bone conduction BAER testing in canines? Does the average amplitude of Wave V of a

bone-conducted brainstem auditory evoked response (BAER) differs between the two test groups? It was hypothesized that there would be a positive correlation between head size and Wave V latency and that no significant difference would be found between the amplitude of Wave V of small dogs and of large dogs.

Data were collected and analyzed from twenty dogs: ten small dogs and ten large dogs. Head size was calculated using two measurements taken using a caliper. An air conduction BAER screening was performed on each dog prior to testing to confirm normal auditory status. Bone conduction BAER waveforms were obtained and replicated for each subject. Absolute peak latencies and peak-to-trough amplitudes were analyzed for Wave V for each subject. There was an observable difference in Wave V latencies between the groups, but it was not found to be statistically significant when a Mann-Whitney U-test was performed. A positive correlation (r = 0.4929) was found between head size and Wave V latency. A difference between the average Wave V amplitudes for each group was observed. This difference was found to be statistically significant along with a negative correlation (r = -0.5789) between head size and Wave V amplitude.

It was hypothesized that these findings relate to the differences in anatomical dimensions; a longer auditory pathway from the cochlea to the brainstem would therefore result in longer transmission times of the electrical signal, manifesting in longer peak latencies of Wave V. Similarly, smaller anatomical dimensions result in the recording electrodes to be closer in proximity to the source of the electrical potential in the brainstem. It was suspected that this is responsible for the differences seen in Wave V amplitude, as the voltage of the electrical potential decreased with increased distance between the source and recording electrode (Atcherson & Stoody, 2012).

Future studies should be conducted with larger sample sizes to replicate and further validate these findings.

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LIST OF ABBREVIATIONS

Abbreviation	Description
ABR	Auditory Brainstem Response
BAER	Brainstem Auditory Evoked Response
CKCS	Cavalier King Charles Spaniel
CN	Cranial Nerve
CT	Computed Tomography
dB	Decibels
FETCHLAB	Facility for Education and Testing of Canine Hearing and Laboratory for Animal Bioacoustics
fMRI	Functional Magnetic Resonance Imaging
IACUC	Institutional Animal Care and Use Committee
JCIH	Joint Committee on Infant Hearing
MRI	Magnetic Resonance Imaging
nHL	Normal Hearing Level
OFA	Orthopedic Foundation for Animals
PSOM	Primary Secretory Otitis Media
SPL	Sound Pressure Level
UNC	University of Northern Colorado

CHAPTER I

STATEMENT OF THE PROBLEM

Brainstem auditory evoked response (BAER) testing is the primary diagnostic tool used by veterinarians and animal audiologists to assess hearing ability in canines. It is the only test recognized and accepted by the Orthopedic Foundation for Animals (OFA), a non-profit organization whose purpose is to fund research and maintain a database of hereditary diseases in dogs, including congenital deafness. The test is designed to measure the auditory nerve and brainstem's electrical activity in response to a sound stimulus (Scheifele & Clark, 2012). Brainstem Auditory Evoked Response testing is typically conducted using insert earphones to present the stimulus through air conduction. Air conduction BAER testing evaluates how well the structures of the auditory pathway are performing, from the external ear to the brainstem. However, the response can also be assessed utilizing bone conduction. This method of presentation provides an estimate of the cochlea's response to sound with minimal contribution of the outer and middle ear.

Both air and bone conduction BAER testing should be utilized for a comprehensive audiologic examination. Stimulating the cochlea via bone conduction allows for assessment of the sensory and neural components of the auditory pathway. Middle ear pathologies, such as otitis media, that could result in a conductive hearing loss in canines can elicit an abnormal BAER result when testing through air conduction. In this case, bone conduction BAER should be utilized to further evaluate the auditory system to determine if the structures beyond the middle ear are affected or contributing to the abnormal results.

Air conduction BAER has been studied repeatedly in canines such that there is normative data published to facilitate interpretation of the test results (Scheifele & Clark, 2012). However, the literature is limited when it comes to bone conduction BAER. Munro, Paul, et al. (1997) published normative data for bone conduction results in canines and speculated that variations in latencies of the waves in the BAER waveform could be attributed to differences in head size among the breeds tested. Kemper et al. (2013) found that results for air conduction BAER testing were not clinically impacted by head size or breed. Despite the findings of Kemper et al. for air-conducted stimuli, it is possible that the physiologic differences inherent in bone-conducted testing will affect the latencies of the BAER. Further investigation into the effects of head size on bone conduction BAER results has not yet been performed. Understanding the influence of head size on bone conduction BAER results would improve the accuracy of interpretation of such results.

Summary

Currently, the literature is limited in the area of bone conduction BAER testing in canines. The majority of the current literature focuses on BAER waveforms produced from air conduction stimuli. There are no published studies that evaluate the effect of canine head size in bone conduction brainstem auditory evoked responses, though some variability in waveform latencies between large and small dogs has been observed by Munro, Paul, et al. (1997) when testing via bone conduction. The goals of the current study are to further evaluate the effect of head size on bone conduction BAER in canines and to contribute additional data to further understanding and interpretation of BAER waveforms in canines.

Research Questions

Q1 What effect does head size have on the absolute latency of Wave V for bone conduction BAER testing in canines?

Q2 Does the average amplitude of Wave V of a bone-conducted brainstem auditory evoked response (BAER) differ between the two test groups?

Hypotheses

H1 The absolute latency of Wave V on the bone conducted BAER waveform will increase proportionally as the subject's head size increases.

H2 There will be no significant variance of average Wave V peak-to-trough amplitudes with varying head size for bone conduction BAER testing in canines.

CHAPTER II

REVIEW OF THE LITERATURE

Auditory Brainstem Response Overview

The auditory brainstem response (ABR) can be described as a series of averaged synchronous neural responses that are generated by the auditory nerve and brainstem auditory pathway in response to acoustic stimulation (Musiek & Baran, 2016). Such responses occur within 10 milliseconds of stimulus onset. This non-invasive procedure records the electrical response along the auditory pathway in response to auditory stimuli. The response is plotted as a waveform with seven individual peaks, labeled Wave I through VII (Jewett & Williston, 1971). Each resulting wave corresponds to a specific anatomical structure along the brainstem auditory pathway with Wave I being the most distal location (Jewett & Williston, 1971). In humans, Wave I originates from the distal portion of cranial nerve (CN) VIII as the nerve fibers depart from the cochlea while Wave II is generated by the proximal portion of CN VIII where it enters the brainstem. Researchers suggest that Wave III is produced at the level of the pons in or near the cochlear nucleus. The neural generators that contribute to Wave IV are poorly understood, however the current literature suggests the superior olivary complex as a main contributor. The most widely accepted origin for Wave V is the lateral lemniscus (Møller, 2013).

Information regarding the origins of Waves VI and VII is limited, however the inferior colliculus is currently suggested as the primary generator involved (Møller, 2013). The ABR can be used to estimate a patient's hearing thresholds, or as a neurodiagnostic tool (DeBonis & Donohue, 2004). When an ABR is done on an animal of any species, it is referred to as the

brainstem auditory evoked response (BAER). One method for testing the entire auditory pathway is the brainstem auditory evoked potential (BAER) test, which presents acoustic stimuli to the ear while measuring electrical activity of the nervous system in response to the stimuli (Scheifele & Clark, 2012; Webb, 2009).

Physiology of Bone Conduction

The human ear is comprised of three parts: the outer ear, the middle ear, and the inner ear. When considering how humans and other mammals hear, it is typically described in reference to air conduction. Hearing via air conduction consists of the pinna of the outer ear funneling sound waves into the ear canal which then vibrate the tympanic membrane (Dallos, 1973). This motion of the tympanic membrane initiates a vibration of the three ossicles within the middle ear: the malleus, the incus, and the stapes. The footplate of the stapes articulates with the oval window of the cochlea, which is the sensory organ of hearing that comprises the inner ear (Dallos, 1973). As the oval window moves, the fluid within the cochlear duct becomes displaced. This displacement causes the basilar membrane to move, which then shears the stereocilia atop the hair cells that sit along the basilar membrane. Ion channels are activated when the stereocilia are sheared, initiating a response which then sends the signal to the cranial nerve VIII to begin its journey to the auditory cortex (Dallos, 1973). However, the air conduction pathway is not the only avenue.

The cochlea is embedded deep within the temporal bone of the skull (Pickles, 1982). Due to its placement, the cochlea may also be stimulated via bone conduction by directly vibrating the skull. The bone conduction pathway may be examined more closely by describing the three routes that a stimulus can travel via bone conduction. The first and primary route of bone conduction is referred to as labyrinthine bone conduction, where the bones of the human skull

vibrate in various patterns depending on the frequency of the stimuli presented. At lower frequencies, such as 200 hertz (Hz), the skull collectively vibrates anteriorly to posteriorly. The vibratory pattern changes as the frequency approaches 800 Hz. At this frequency, the rostral portion of the skull moves anteriorly while the dorsal portion moves posteriorly (Stenfelt, 2011; Zemlin, 1981). Changes in the vibration pattern continue as the frequency increases. A frequency of about 1500 Hz will initiate a vibration pattern similar to that of 800 Hz, with the addition of the lateral portions of the skull vibrating medially, much like the vibration pattern of a bell (Zemlin, 1981). Regardless of frequency, the vibratory motion of the skull via this bone conduction route will displace the fluid within the cochlea to stimulate the hair cells.

The second bone conduction route, known as the inertial route, involves the ossicles of the middle ear. The walls of the middle ear vibrate along with the temporal bone, but since the ossicles are suspended, they remain relatively stationary while this movement occurs due to their inertia. Consequently, the oval window and the fluid within the cochlea are displaced as the ossicles exert a force in the opposite direction of the vibratory motion (Stenfelt, 2011; Zemlin, 1981).

The final route, known as the osseotympanic route, involves the temporomandibular joint, which is located just below the ear canal. The mandible is not directly connected to the bones of the skull and thus cannot vibrate cohesively in the same pattern when the bone is stimulated by a sound vibration. Vibratory movement does occur within the mandible, but it is considered to be out of phase with the vibrations of the skull. Due to this dys-synchronous vibration, the cartilage of the ear canal is displaced in such a manner that the air within the ear canal can vibrate, creating a pressure wave which then moves the tympanic membrane and is perceived as sound (Zemlin, 1981).

Forehead versus Mastoid Oscillator Placement in Human Subjects

Before the sound vibrates the bones of the skull via any of the aforementioned bone conduction routes, it must first pass through the skin and underlying tissue. This has caused some debate on which placement on the patient's skull is ideal for bone conduction testing. For humans, placement on the mastoid process is the most typical location of the bone oscillator during bone conduction testing. However, Békésy and Bárány, as reported by Studebaker (1962), have suggested that this placement may be problematic. Instead, placement of the oscillator on the forehead may yield better results (Studebaker, 1962). It has been suggested that mastoid placement will involve a higher variability of the skin and tissue among subjects, thus causing variability in thresholds as thicker skin and tissue will attenuate the sound, particularly frequencies above 2000 Hz, as it travels through to the skull (Stenfelt, 2011). Skin and tissue anatomy at the forehead is considered to be more consistent among individuals. The oscillator may also shift and/or contact the outer ear when placed on the mastoid, leading to unintentional hearing via air conduction (Studebaker, 1962). Twenty subjects were included in a 1962 study by Studebaker to examine the variations in threshold that could be produced by changing the bone oscillator placement. In unoccluded ears, the average difference in decibels (dB) between thresholds obtained via forehead placement and mastoid placement indicate that mastoid placement results in lower thresholds, particularly at lower frequencies. At 500 Hz, the difference between forehead and mastoid placement thresholds was 14.8 dB whereas the difference at 4000 Hz was 5.2 dB (Studebaker, 1962). This finding is consistent with information reported by Seo et al. in a 2018 review.

Similarly, it was found that the variation of a single threshold measure among the 20 test subjects was lower when tested using the forehead placement location at lower frequencies. The

standard deviations at 250 Hz were 3.93 dB and 5.15 dB for the forehead and mastoid placements, respectively. As frequency increased to 4000 Hz, the standard deviation fell to 4.24 dB for mastoid placement and rose to 5.95 dB for forehead placement (Studebaker, 1962). It was concluded that measurements obtained via forehead placement exhibited less variability than those obtained via mastoid placement. Likewise, the forehead yielded thresholds that were less affected by middle ear pathologies when compared to thresholds obtained at the mastoid (Studebaker, 1962). Seo et al. (2018) reported that when using bone conduction for ABR specifically, infant subjects were more sensitive to oscillator placement than adults. A delayed latency of Wave V was observed in the ABR response of infants when the oscillator was placed at the frontal bone as compared to placement on the temporal bone. Seo et al. also suggest that the density and thickness of the cranial bones can contribute to ABR responses and may also influence the placement of the oscillator.

Clinical Uses of Bone Conduction

Bone conduction testing is an integral part of auditory assessment since it directly assesses the function of the inner ear by bypassing the outer and middle ear, as described in the previous section. The original bone conduction test utilized tuning forks to perform the Weber and Rinne tests in the 19th century (Stenfelt, 2011). With developments of a bone conduction transducer coupled to an audiometer, bone conduction became an invaluable diagnostic tool to distinguish a conductive hearing loss that affects the outer or middle ear from a sensorineural hearing loss where the lesion would exist at the cochlea or higher up in the central auditory pathway (Stenfelt, 2011). A conductive loss is characterized by what is called an “air-bone gap,” or a difference between hearing thresholds obtained via air conduction and thresholds obtained

via bone conduction methods, with bone conduction thresholds being lower (better) than air conduction thresholds (Stenfelt, 2011).

The bone-conducted ABR is routinely used in some clinics for testing infants and small children when a conductive loss may be suspected. The Joint Committee on Infant Hearing (JCIH) highlights the value of bone conduction testing in a comprehensive auditory assessment test battery in order to distinguish between a conductive and sensorineural hearing loss (Hatton et al., 2012). Hatton et al. (2012) suggested that the bone-conducted ABR is a reliable tool to not only determine cochlear function but also to estimate or assist in determining the degree of sensorineural impairment by way of presenting stimuli at higher intensity levels.

Comparing Air and Bone Conduction Responses in Humans

In comparing brainstem responses in humans that were evoked by air and bone conduction, Seo et al. (2018) found that the two responses should be similar in morphology, latency, and amplitude when the stimuli are presented at the same intensity level for both air and bone conduction presentations in patients with normal hearing. However, it has been suggested that latencies of waves obtained by bone conduction can be about 0.16 to 0.88 milliseconds longer than those obtained by air conduction in normal hearing subjects (Seo et al., 2018). Cornacchia et al. (1983) found that in normal hearing subjects, bone-conducted ABRs exhibited latencies that were longer than air-conducted ABRs by an average of 0.56 milliseconds in adults and 0.67 milliseconds in infants. A longer traveling wave delay or propagation delay and low-pass filtering of the bone oscillator in skull vibration are suspected to contribute to this effect (Seo et al., 2018). The opposite was found by Cornacchia et al. (1983) and Yang et al. (1987) in infants, where the latency of Wave V is shorter in waveforms obtained using bone-conducted clicks. This is attributed to the maturation and changes that occur in the skull with age.

If a conductive hearing loss exists, the bone-conducted response should be similar to that of a normal-hearing response whereas the air-conducted response will display prolonged latencies for all waves of the response (Seo et al., 2018).

Canine Auditory System and Anatomy

As in humans and other mammals, the canine auditory system consists of the outer, middle, and inner ear. The most prominent structure of the outer ear, the pinna, varies in size and shape among breeds of dog. Some dogs have naturally erect pinnae while others have long, pendulous pinnae (Njaa et al., 2012). The pinnae are structures made-up of auricular cartilage covered by hair and skin, which contains both sweat glands and sebaceous glands. The pinnae are flexible such that they can move easily (Cole, 2010). As with human pinnae, the canine pinnae's primary functions are to aid in localization and transmitting sound to the more proximal components of the auditory system (Njaa et al., 2012). There are numerous muscles that control the orientation of the pinnae to facilitate localization. The main muscular groups include the rostroauricular muscles and the caudoauricular muscles, along with one ventroauricular muscle (Cole, 2010). To a certain degree, the pinnae serve to protect the ear canals, which open dorsolaterally and are surrounded by the cartilage of the pinna, including the tragus (Njaa et al., 2012). Unlike the S-curve shape of the human ear canal, the ear canal of the dog involves a right angle turn that separates the canal into two portions: the vertical canal and the horizontal canal. The vertical canal veers medially slightly above the level of the tympanic membrane. The remaining portion of the ear canal is considered the horizontal canal. At the point where the canal deviates, a prominent cartilaginous ridge, called Noxon's ridge, marks the transition from vertical to horizontal canal (Cole, 2010).

The horizontal canal terminates at the tympanic membrane. The size of the tympanic membrane is highly correlated to the size of the dog. In 1983, Heffner found that the tympanic membrane varied in size from 30 mm^2 to 55.3 mm^2 among dogs ranging from 4.3 kg to 45.5 kg in weight. The tympanic membrane includes two regions: the pars tensa and the pars flaccida. The pars flaccida typically lies flat with a pink color in healthy dogs. A bulging pars flaccida could indicate an infection in the middle ear, but could also be present with no underlying pathology (Cole, 2010). The main portion of the tympanic membrane, the pars tensa, remains thin yet tough, with a translucent gray color (Cole, 2010). Beyond the tympanic membrane lies the air-filled middle ear space, also referred to as the tympanic cavity (Cole, 2010; Njaa et al., 2012).

As with humans, the middle ear cavity houses three small ossicles: the malleus, the incus, and the stapes. The manubrium of the malleus articulates with the tympanic membrane while the head of the malleus articulates with the body of the incus to form the incudomalleolar joint. The lenticular process of the incus then hinges with the head of the stapes at the incudostapedius joint (Njaa et al., 2012). Working as a chain, these ossicles move in response to vibrations of the tympanic membrane, carrying the vibration to the footplate of the stapes. The stapes then articulates with the oval window at the vestibule of the inner ear.

The petrous portion of the temporal bone protects the cochlea, which is housed in a bony labyrinth (Cole, 2010). The bony labyrinth consists of three semicircular canals, the spiral cochlea, and the vestibule which sits between them (Cole, 2010). The cochlear duct, which lies within the spiral cochlea of the bony labyrinth, houses the organ of Corti, tectorial membrane, vestibular membrane, and sensory cells bathed in endolymph. As the ossicles interact with the oval window, the perilymph within the scala tympani and scala vestibuli becomes displaced,

resulting in shearing of the hair cells along the basilar membrane in the organ of Corti. This shearing action causes ion channels to open, thus depolarizing the hair cells, which then in turn transmit the electrical signal to the cochlear branch of the vestibulocochlear nerve via synapses at the bases of the hair cells. Once in the nervous system, the electrical signal then travels to the brainstem and ultimately the auditory cortex (Cole, 2010). Damaged hair cells can inhibit the ability to generate an electric signal, resulting in a sensorineural hearing loss (Strain, 2012; Webb, 2009). Functional magnetic resonance imaging (fMRI) showed illuminations of the superior olivary nucleus, lateral lemniscus, and internal capsule along with voxels in the auditory cortex when presenting a group of Beagles with auditory stimuli, suggesting that those structures are prominent components of the canine auditory pathway (Bach et al., 2016).

A significant difference between the auditory system of humans and canines lies in the structure of the cochlea. In humans, the cochlea consists of about 2 ¾ turns. However, the cochlea in a dog has approximately 3 ¼ turns (West, 1985). West found that upper and lower limits of hearing also varied between humans and dogs. In humans, the lower limit at 30 dB sound-pressure level (SPL) was 110 Hz, compared to that of a dog's at 200 Hz. The upper limit at 30 dB SPL for humans and dogs were measured at 16,000 Hz and 36,000 Hz, respectively (West, 1985). At 60 dB SPL, the frequency range of human hearing was 29 to 19,000 Hz while the range for canines at 60 dB SPL was 64 to 44,000 Hz (West, 1985).

Furthermore, upon examining differences in thresholds between humans and dogs using behavioral measures, Lipman and Grassi (1942) found that auditory thresholds for humans and dogs were the same at 125 and 250 Hz when utilizing behavioral audiometry. However, as frequency increased, the dogs' thresholds surpassed those of the humans. At 1000 Hz, dogs'

threshold surpassed the humans' by 13 dB, and 19 dB at 4000 Hz, suggesting that dogs have better hearing abilities than humans in the higher frequencies (Lipman & Grassi, 1942).

Heffner (1983) performed a study on five dogs of various breed and size where their auditory thresholds were determined through behavioral measures. It was determined that the size of the dog, the interaural distance, or the area of the tympanum had no significant effect on the auditory threshold, regardless of frequency. It was seen that there was less variability among subjects at high frequencies, particularly 32,000 Hz and above (Heffner, 1983).

Lastly, dogs are susceptible to different types and degrees of hearing loss or deafness, just as humans are. Heffner (1983) reports that dogs typically have a hearing range from 67 Hz to 45,000 Hz, whereas humans have a typical range of 29 Hz to 19,000 Hz, according to West (1985). Dogs can experience unilateral or bilateral deafness, noise-induced hearing loss, progressive hearing loss, peripheral deafness, or central deafness--all of which are present in human patients (Strain, 2012). Heredity or acquired etiologies can cause peripheral deafness, or pathologies that affect the outer ear, middle ear, or cochlea. Sensorineural peripheral deafness of the cochlea can correspond with lack of pigment, anoxia, presbycusis, trauma, or otitis interna. Conductive peripheral deafness can result from atresia, otitis externa, otosclerosis, primary secretory otitis media (PSOM), or cerumen impaction (Strain, 2012). Dogs can experience symptoms seen in humans, such as tinnitus or hyperacusis (Strain, 2012). With hyperacusis, or increased sensitivity to sounds, specific causes remain undiscovered but researchers suspect noise-induced hearing loss. Often times, dogs with reported hyperacusis have normal BAER results (Strain, 2012). Dogs have been reported to exhibit objective tinnitus, which is typically high-frequency sound generated by the ear that can be heard via stethoscope (Strain, 2012).

Brainstem Auditory-Evoked Response Procedures

When testing humans, surface electrodes that are placed on the patient's skin are used to measure the response, but needle electrodes are used when testing animals. When testing a non-sedated animal, a topical anesthetic, such as 2.5% lidocaine and 2.5% prilocaine cream, is applied to the placement areas of the three subdermal electrodes. The electrodes are not necessarily painful, but the local anesthetic can provide maximum comfort to the animal during the procedure and provide the animal's owner some peace of mind. The subdermal electrodes measure 0.4mm in diameter and 13mm in length and are placed in three locations. The positive, non-inverting electrode is placed on the vertex (Cz). The negative, or inverting, electrode is placed anterior to the tragus (Ai) of the test ear while the non-test ear is fitted with the ground electrode (Ac), also just anterior to the tragus. The electrodes are then connected to the computer-based equipment via the electrode box, also known as a preamplifier. Impedances of the electrodes are checked using the electrode box and should be re-checked before each test recording. Testing should be run using the guidelines and recommendations outlined in the next section. Each intensity level must be tested twice to establish replication criteria. The Wave V peak and/or trough must be identifiable and within 0.1 milliseconds across the two waveforms (Scheifele & Clark, 2012). When using BAER in threshold estimations, the lowest intensity level that produces an identifiable and repeatable Wave V determines threshold (Munro, Paul, et al., 1997; Scheifele & Clark, 2012).

Brainstem Auditory Evoked Response Instrumentation and Stimulus Parameters

Specific equipment is required in order to obtain a canine brainstem auditory-evoked response (BAER), although it is the same as that for humans with the exception of the type of electrodes. The equipment is computer-based and components can be classified as recording or

stimulus components. The recording equipment includes subdermal recording electrodes, a display screen, differential preamplifier, and a signal averager. The stimulus generator and transducer are classified as stimulus components (Scheifele & Clark, 2012). As suggested by Scheifele and Clark when testing canines, the amplifier should be set to record in microvolts and have an absolute gain of 100,000 to 150,000. A high-pass filter set at 300 Hz and a low band-pass filter set at 1500 Hz are also recommended. The signal averager, used to isolate the brainstem response from ambient electrical noise, is recommended to run 1,000 to 2,000 sweeps at each stimulus level to ensure an accurate representation of activity in the central nervous system (Scheifele & Clark, 2012).

A 100-microsecond broadband click stimulus with 12,000 Hz bandwidth power is typically employed to acquire a BAER. The click contains energy in the range of 500 to 4000 Hz, but only effectively stimulates the 2000 to 4000 Hz region of the cochlea in both humans and animals (Scheifele & Clark, 2012). Most equipment is limited in that the maximum frequency it can test is 14,000 Hz. The stimulus can be set to different polarities including condensation, rarefaction, and alternating polarity (Scheifele & Clark, 2012).

For canines, a stimulus rate of 33.3 clicks per second was found to minimize testing time without compromising the quality of the BAER waves (Scheifele & Clark, 2012). Stimulus intensity can play a large role in the BAER waveform. The dB scale of nHL is not acceptable for diagnostic use in canines as it refers to a normalized hearing level in humans. Instead, Scheifele and Clark (2012) recommend using dB peSPL units where the reference for a 0 dB peak sound pressure is 20 µPa. “For any sound, this reference is equal to 20 times the logarithm to the base 10 of the ratio of the pressure of the sound measured to the reference pressure; the typical reference for 0 dB root mean square sound pressure level (SPL) is 20 µPa” (Scheifele & Clark,

2012, p. 1246). When using BAER in diagnostic cases, Scheifele and Clark (2012) recommended testing the following intensities: 70 dB peSPL, 80 dB peSPL, 90 dB peSPL, 102 dB peSPL, and 116 dB peSPL, presented in ascending order. When testing with bone conduction click stimuli, there are no suggested guidelines for parameters, though condensation or alternating polarity is recommended.

Current Brainstem Auditory Evoked Response Data for Air and Bone Conduction in Dogs

The auditory brainstem response (ABR) is accepted as a valid and reliable method for evaluating hearing abilities in humans. Unlike pure tone audiometric testing, the ABR requires no behavioral response from the patient, making it ideal for patients who are unable to respond to the more traditional behavioral hearing tests. Due to the ability to test patients with a non-behavioral procedure, veterinary practices can employ the ABR when testing animal patients (Munro, Paul, et al., 1997). When using the ABR electrodiagnostic test on animals, it is referred to as a brainstem auditory evoked response, or BAER (Scheifele & Clark, 2012). In addition to not requiring a behavioral response, utilization of the BAER test on sleeping or sedated animals does not compromise the test reliability (Munro, Shieu, et al., 1997).

Munro, Paul, et al. (1997) collected normative values for bone conduction BAER testing. Forty dogs were used, including 20 Dalmatians and 20 Jack Russell terriers. Dogs included in this study were in healthy condition as determined by a veterinarian and had normal otoscopy. None of the subjects had a history of ear disease or any concerns about hearing at the time of testing. Veterinarians involved in the study administered medetomidine hydrochloride to sedate canine subjects for testing. The veterinarians also monitored the vital signs of all subjects during the testing procedure. A 0.1 millisecond square wave click stimulus of alternating polarity was delivered at a rate of 11.1 clicks per second, while utilizing a bandpass filter from 100 to 3000

Hz. Thresholds were determined by decreasing the stimulus level in successive 10 dB steps from 30 dB normal hearing level (nHL) and evaluating the morphology of the response waveform, looking specifically for a well-defined Wave V. The bone oscillator was placed at the vertex in this study. Threshold was determined to be at the lowest level at which a Wave V was identifiable. Two different methods of application of the bone vibrator were tested: applying a 500-gram weight to the bone vibrator and holding the bone vibrator against the dog by hand with firm pressure. The authors did not find a significant difference between the two applications. Jack Russell terriers had a shorter latency for Wave V when compared to the Dalmatians. The researchers speculated that the smaller head size and smaller brainstem dimensions of the Jack Russell terrier contributed to this difference. In both breeds, however, the latencies for all waves were found to be closely in agreement with air conduction BAER results for the same dog (Munro, Paul, et al., 1997).

In another study investigating the effect of head size in air-conduction BAER responses, Munro, Shiu, et al. (1997) found that the absolute latency of Wave V was 0.3 milliseconds longer in Dalmatians than it was in Jack Russell terriers, but this correlation was not found to be statistically significant. Similarly, Kemper et al. (2013) evaluated 43 dogs of various breeds and determined that neither breed nor head size had a clinical impact on wave latencies or morphology of air-conducted BAER results. Head size was determined using a caliper to measure the distance between the non-inverting and inverting electrodes as measured from the temporal bone portions of the temporomandibular joint on each side of the head, referred to as the “tymp-to-tymp” measurement (Kemper et al., 2013). A secondary measurement was taken from the top of the head to the occipital bone, referred to as the “occ-to-stop” measurement.

Below is the equation to calculate head size using the two measurements as published by Kemper et al. (2013):

$$\text{Head size} = \sqrt{tymp - to - tymp/2} + \sqrt{occ - to - stop/2}$$

Although the dB scale of nHL is used in humans, the data suggest that this reference level may be appropriate for dogs to determine bone conduction thresholds. Munro, Paul, et al. (1997) found that the average bone conduction threshold in dogs was close to 0 dB nHL.

To further determine an accurate procedure for performing bone conduction BAER testing in dogs, Strain et al. (1993) investigated the effects of various bone vibrator placements. Performing testing on 16 healthy adult Beagles from a university veterinary school population, the following placements of the bone vibrator were tested: vertex, midline caudal to the interorbital line, mastoid process, caudal-ventral body of mandible, against the gingiva of the first upper premolar, and zygomatic arch. Handheld placement with firm pressure was employed to test all placements. The researchers described the amount of pressure as being just under the maximal pressure the dog could tolerate. They first performed air conduction BAER testing on each dog to later compare to results of bone conduction testing. The researchers found that condensation polarity click stimuli at a rate of 11.4 clicks per second resulted in the best peak definition on the waveform when compared to rarefaction and alternating polarities. A bandpass filter was applied from 150 to 3000 Hz. Latency and amplitude values for Waves I, II, III, and V were collected at stimulus levels of 95 dB nHL, 75 dB nHL, and 55 dB nHL. Strain et al. (1993) found that the absolute latencies for bone conduction were longer than those seen in air conduction BAER results when the latencies were corrected to account for transit time of the air-conducted stimuli. Data tables included standard deviation values for absolute latencies for each wave as measured by both air and bone conduction. Strain et al. (1993) concluded that the ideal

placement of the bone vibrator was on the mastoid process, followed by the mandible and zygomatic arch.

The age at which a dog can be tested may limit the utilization of bone conduction BAER testing. Air conduction BAER testing can be performed as early as 5 weeks of age, but because bones are not fully developed at birth, the incomplete ossification and incomplete closure of cranial bone sutures could hinder the results of bone conduction testing (Strain et al., 1993). Bone conduction BAER results for adult dogs aged one year or older persist as the only reported norms at present.

Scheifele and Clark (2012) suggested that although there are accepted normative values for bone conduction BAER testing, the parameters used to collect these data are inconsistent among studies. There remains a lack of agreement on universally accepted clinical norms. Consequently, duplication of published studies becomes difficult and little support exists to accurately diagnose based solely on waveform morphology. In order for the procedure to achieve standardization, the click presentation rate, bandpass filter settings, and use of a standard dB reference level, such as dB peak-equivalent sound pressure level (peSPL), must remain consistent and agreed upon. At present, handheld mastoid placement, a click rate of 11.4 per second, and a bandpass filter from 150 to 3000 Hz yield reliable results across studies in both dogs and cats (Strain et al., 1998). Despite a lack of standardization, the BAER test remains the only test accepted by the Orthopedic Foundation for Animals (OFA) to document hearing impairment or deafness in dogs, suggesting that the test persists as reliable (Scheifele & Clark, 2012).

Rationale for Performing Bone Conduction Brainstem Auditory Evoked Response in Dogs

Hearing loss or deafness in canines can be due to a variety of factors. Genetics, age, pigmentation, ototoxicity, and infections such as otitis media are among these contributors. Inherited deafness can be described as (a) congenital or late onset, (b) cochlea-saccular, neuroepithelial, or other, and (c) sensorineural or possibly conductive (Strain, 2015). Hereditary deafness is more prevalent in some breeds and in most cases is sensorineural. Some breeds known for genetic deafness include Dalmatians, Border Collies, Australian Shepherds, Doberman Pinschers, Flat-coated Retrievers and Pointers. In a study by Schmutz (2014), a group of 216 Border Collies were examined for late-onset deafness utilizing the BAER test. Researchers did not state how they defined deafness or how the BAER results were interpreted. Of the six dogs aged 12 years or older, four were deaf in one or both ears, possibly suggesting that geriatric hearing loss is common in this breed (Schmutz, 2014). Schmutz proposed that adult onset deafness follows an autosomal dominant pattern of inheritance. Another potential cause of sensorineural hearing loss or deafness in dogs is ototoxicity. Research on the topic remains limited in canine subjects, however recent studies suggest that dogs receiving treatments involving cisplatin, aminoglycosides, and diuretics are susceptible to hearing loss due to ototoxicity (Oishi et al., 2012).

Bone conduction BAER testing gains relevance when determining whether or not there is a conductive component to the hearing loss. The bone-conducted stimuli largely bypass the middle ear cavity and more directly stimulate the inner ear (Scheifele & Clark, 2012). When compared to air conduction BAER results for the same animal, bone conduction BAER results can assist in determining the site of lesion and can lead to a more accurate diagnosis. Otitis media remains a primary cause of conductive deafness or hearing loss in dogs; one type in

particular is especially damaging. Primary secretory otitis media (PSOM) is commonly seen in Cavalier King Charles Spaniels (CKCS) and is characterized by mucus in the middle ear cavity (Scheifele & Clark, 2012). Current research proposes a correlated relationship between PSOM and the nasopharyngeal conformation found in brachycephalic breeds, such as the CKCS and Boxer (Cole, 2012). In the CKCS breed specifically, thickness of the soft palate also plays a role in the prevalence of otitis media with effusion, including PSOM. A bulging pars flaccida portion of the tympanic membrane seen during otoscopy by a veterinarian is the primary means to diagnose PSOM in dog. However, many CKCS with PSOM have presented with a flat pars flaccida. In such cases, additional radiographic imaging, such as computed tomography (CT) scans or magnetic resonance imaging (MRI), must be performed to confirm the diagnosis (Cole, 2012). Harcourt-Brown et al. (2011) revealed some effects of the presence of PSOM on the air-conduction BAER in CKCSs, including an elevated threshold. Conductive hearing losses will shift the slope of a latency-intensity function, which was seen by Harcourt-Brown et al. when examining the effects of PSOM.

Adding bone conduction BAER to the test battery for the evaluation of PSOM would assist in making an accurate and quick diagnosis of hearing sensitivity in canines. A case example described by Scheifele and Clark (2012) involving a CKCS with PSOM depicted a flat BAER tracing when testing using air-conducted stimuli at 70 dB peSPL. When the same dog was tested using bone-conducted stimuli at the same intensity level, the typical BAER waveform was restored, suggesting that the hearing loss was conductive (Scheifele & Clark, 2012). Without bone conduction BAER waveforms, this dog may have been incorrectly diagnosed or the PSOM left medically untreated. Applications of bone conduction BAER include diagnostic testing and screening, just as with air conduction BAER. As seen with the dog in this case report, recording

a BAER with the two different stimulus transducers, air and bone, provided additional information regarding the pathology and site of lesion. During screenings, bone conduction BAER could be performed if air conduction results were questionable.

Summary

While multiple studies have been performed that examine the bone-conducted brainstem auditory evoked response in canines (Munro, Paul, et al., 1997; Strain et al., 1993), a study specifically designed to investigate the effects of head size on the bone-conducted response remains to be completed. Kemper et al. (2013) suggest that head size does not have a significant effect on the BAER when performed with an air-conducted stimulus. Behavioral audiometric thresholds in canines as measured by Heffner (1983) also indicated no significant effect of size among subjects. However, Munro, Paul, et al. (1997) reported a consistent measurable difference in Wave V latency between a group of Dalmatians and a group of Jack Russell terriers during bone conduction BAER testing. The researchers speculated that these differences may be related to the subjects' head size. Although there is no evidence to suggest that air-conducted BAER latencies are affected by head size, the physiologic differences that exist when testing via bone may have an influence on the latencies of the BAER. The purpose of the proposed study is to assess any potential effects of head size on the bone-conducted BAER in canines, which would be especially crucial in cases where bone-conducted BAER testing is performed in dogs with a suspected conductive hearing loss or dogs who cannot be tested with air-conducted stimuli.

CHAPTER III

METHODOLOGY

The current study was designed to evaluate the effect of head size on bone conduction BAER waveforms in canines by comparing Wave V latency and amplitude between two groups: dogs with small-sized heads and dogs with large-sized heads. This study was conducted following Institutional Animal Care and Use Committee (IACUC) approval.

Subjects

A total of twenty dogs of various breeds were included in testing. Three dogs were excluded from the study. Two dogs exhibited significant stress during air conduction testing and were excluded so as to not cause them unnecessary stress or discomfort. A third dog did not pass the air-conduction BAER screening and was excluded as it did not fit the inclusion criteria of having normal hearing. The included dogs were divided into two groups based on head size; ages ranged from 15 to 158 months. One group consisted of ten dogs with small-sized heads, such as Jack Russell terriers, Chihuahuas, or Corgis. The second group consisted of ten dogs with large-sized heads, such as Mastiffs, Rottweilers, or Saint Bernards. The groups in this study were defined by small heads ranging from 8.20 cm to 12.20 cm, and large heads ranging from 15.02 cm to 18.47 cm. Head size was measured utilizing the same method as Kemper et al. (2013). A caliper was used to determine the distance between the inverting and non-inverting electrodes, referred to as the “tymp-to-tymp” measurement, as measured from the temporal portions of the temporomandibular joints on either side of the head. A caliper was also used to measure the distance from the dog’s stop to its occipital bone, referred to as the “occ-to-stop” measurement.

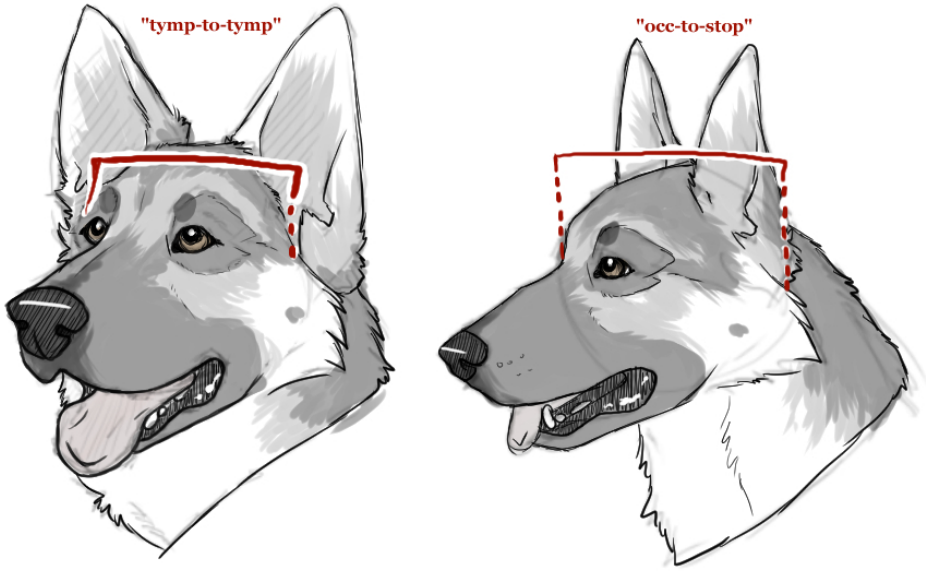
A visual representation of each measurement is shown in Figure 1. The two measurements were then utilized to calculate the head size using the formula described by Kemper et al. (2013):

$$\text{Head size} = \sqrt{(tymp - to - tymp)^2 + (occ - to - stop)^2}$$

Subjects were recruited through flyer advertisements, social media advertisements, and word-of-mouth. Subjects included in the study did not have any current symptoms of otic disorders, such as drainage, excessive debris in the ear canal (cerumen, ear mites, or yeast/bacterial infection), or visible inflammation of the ear canal. Subjects were all neurologically normal and had no history of hearing loss per owner report. All test subjects were in good health at the time of testing, as indicated by a veterinarian wellness check performed prior to testing. The Institutional Animal Care and Use Committee (IACUC) approved testing of animals at the Facility for Education and Testing of Canine Hearing and Laboratory for Animal Bioacoustics (FETCHLAB) at the University of Northern Colorado to ensure that the research was performed ethically and that procedures were conducted in an ethical and humane manner.

Figure 1

Illustration of the "tymp-to-tymp" measurement and the "occ-to-stop" measurement

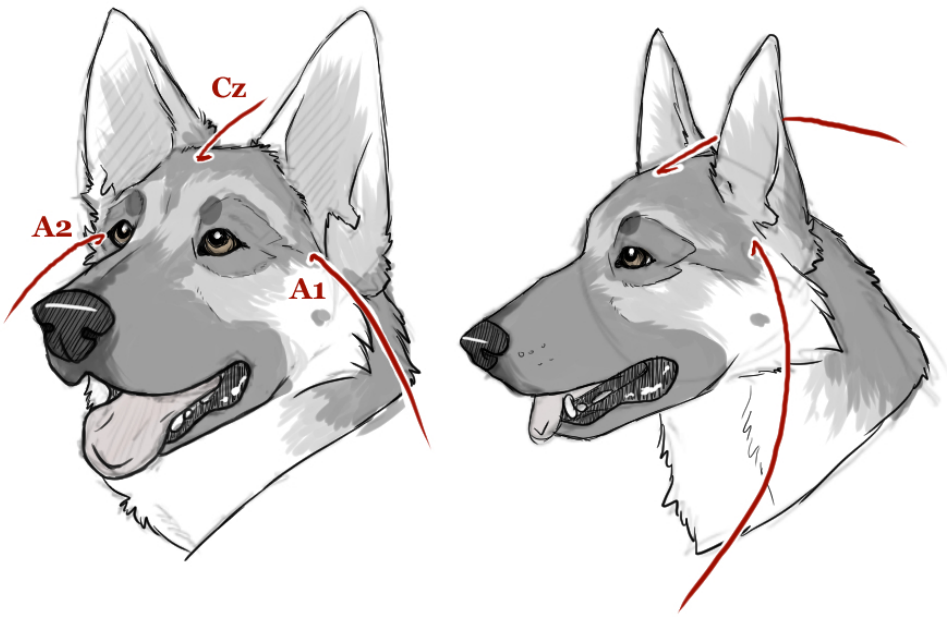


Test Environment, Procedure, and Instrumentation

Testing was performed at Full Circle Veterinary Care in Johnstown, Colorado. The computer-based Intelligent Hearing Systems SmartEP Universal Smart Box with software version 5.2 was used to measure the air and bone conduction BAERs in all subjects. Chemical restraint was not used on any subject. Prior to testing, 2.5% lidocaine and 2.5% prilocaine cream was applied to each of the three placement locations of the subdermal electrodes. Disposable Rhythmlink (product number: RLSND116-1.5) bent subdermal 13mm needle electrodes with a diameter of 0.4mm were used for testing. The ground electrode was placed anterior-inferior to the tragus of the left ear (A1). The negative, or inverting, electrode was placed anterior-inferior to the tragus of the right ear (A2) and the positive, or non-inverting, electrode was placed on the vertex (Cz). A visual representation of electrode montage is shown in Figure 2. During air conduction testing, the inverting and non-inverting electrodes were switched when testing opposite ears. Impedances of the electrodes were checked prior to testing. Satisfactory impedance was defined as 3,000 Ohms or less. A 1-channel recording was used to record all waveforms.

Figure 2

Illustrated electrode montage



An air-conduction BAER screening was performed on all subjects in both ears to confirm normal auditory function on the day of testing. Air-conduction testing was performed utilizing ER-2 insert earphones from Etymotic Research. The foam eartips were inserted into the opening of the vertical portion of the subject's ear canal. Regular (13mm) or small (10mm) sized foam eartips were used to obtain an appropriate fit to the subject's ear canal. The parameters for the air-conduction screening testing consisted of a bandpass filter from 100-1500 Hz and a rarefaction click stimulus presented at 98 dB SPL at a rate of 35.1 clicks per second for 500-1000 sweeps per run. Both ears were tested independently and two waveforms were obtained in each ear to ensure repeatability of Wave V. One dog that did not pass the screening was excused from the study.

Bone-conduction BAERs were obtained using a Radioear B71W transducer with an input impedance of 300 Ohms and a frequency-dependent maximum output range of 109-62 dB SPL

(Intelligent Hearing Systems, 2011). The bone vibrator was applied by hand to the subject's right zygomatic process using the maximal amount of pressure that the subject would comfortably tolerate. Munro, Paul, et al. (1997) found no difference between thresholds obtained by applying the vibrator with firm hand pressure and thresholds obtained by applying the bone vibrator with a 500g weight. The zygomatic process was selected for bone vibrator placement based on findings in the 1993 study by Strain et al. which listed the zygomatic arch as an acceptable placement of the bone oscillator. The ideal placement according to the study is the mastoid process, however, the zygomatic process was selected due to its ease of accessibility on various dogs, overall comfort of the dog, and ability to maintain consist placement and pressure. Subjects were of various breeds and coat types, some with a thick coat around the mastoid process. To avoid any effect of coat thickness on bone vibration stimulation, the zygomatic process was chosen as that is the area where coat length and thickness was most consistent between subjects. Parameters for bone conduction testing utilized a bandpass filter of 30-1500 Hz and an alternating polarity click stimulus presented at a rate of 11.4 clicks per second for 500-1000 sweeps per run. A preliminary pilot study performed through FETCHLAB UNC revealed a more robust Wave V when the stimuli were of alternating polarity. If the waveforms obtained using alternating polarity were poor or unsatisfactory, testing was repeated using a condensation polarity. Two runs were performed on each subject to confirm repeatability of the waveform morphology, particularly that of Wave V. These two waveforms were then averaged together in the software to produce a final waveform for evaluation. The clicks were presented at an intensity of 58 dB nHL. According to the IHS software, dB SPL was unable to be used when testing via the bone vibrator. Additional runs were performed at 38 dB nHL and 18 dB nHL to assist in identifying Wave V as needed. Equipment calibration is such that there is a conversion of 40 when

converting dB nHL to dB SPL. For example, a 58 dB nHL intensity level is calibrated to be equivalent to 98 dB SPL. The primary tester and a secondary tester independently marked absolute latencies and the amplitude for Wave V on the averaged waveform. These values were compared to ensure there was agreement within 0.4 milliseconds among testers regarding the latency and within 0.04 μV for amplitude of Wave V in all waveforms obtained. Absolute latencies, wave amplitudes, and overall waveform morphology was compared between groups and assessed for effects of varying head size. A Mann-Whitney *U*-test was performed to test for differences between groups and the Pearson's correlation coefficient (*r*) was measured to assess the correlation between head size and latency and head size and amplitude. Descriptive analysis was presented for sample characteristics (age and head size) and Wave V amplitude and latency measurements by subject and subject group.

CHAPTER IV

RESULTS

The purpose of the current study was to compare Wave V latency and amplitude between dogs with small-sized heads and dogs with large-sized head to further examine the effect of head size on the bone-conduction BAER waveform in canines.

Data were collected from a total of 20 canine subjects ranging in calculated head size from 8.20 cm to 18.47 cm. The subjects were separated into two groups based on head size: 10 dogs with small head sizes (8.20 cm to 12.20 cm) and 10 dogs with large head sizes (15.02 cm to 18.47 cm). A summary of breed, age, and head size of each canine included in the data analysis can be found in Table 1.

Air conduction BAER responses were obtained from each subject to ensure normal hearing prior to proceeding with bone-conduction. Overall morphology of the waveforms was good with clear, identifiable Wave I and Wave V peaks present and repeatable for all subjects. Mean latency findings for the air-conduction results are summarized in Tables 2 and 3. Mean amplitude findings for air-conduction waveforms are summarized in Table 4.

Table 1

Breed, Age, and Head Size of Each Test Subject

Subject	Bread	Age (months)	Head Size (cm)
1	Labrador Retriever	94	18.47
2	Boxer Mix	40	17.71
3	Siberian Husky	77	17.42
4	Labrador Retriever	90	17.34
5	Shepherd Mix	57	16.98
6	Labrador Retriever	126	16.72
7	Olde English Bulldogge	41	16.02
8	Siberian Husky Mix	55	15.25
9	Berger Picard	15	15.09
10	Australian Cattle Dog	35	15.02
11	Cocker Spaniel Mix	74	12.20
12	Russell Terrier	20	12.02
13	Fox Terrier Mix	78	10.97
14	Chihuahua	58	10.16
15	Miniature Rat Terrier	158	9.59
16	Yorkshire Terrier	152	9.46
17	Chihuahua Mix	44	9.04
18	Chihuahua	56	8.84
19	Miniature Rat Terrier	27	8.65
20	Miniature Rat Terrier	28	8.20

Table 2

Summary of Mean Air-Conduction Latency Findings for the Right Ear

Subject Group	Head Size		Wave I Latency, Right Ear		Wave V Latency, Right Ear		Wave I-V Interpeak Latency, Right ear	
	cm	*SD*	ms	*SD*	ms	*SD*	ms	*SD*
Small Head Size	9.91	1.40	1.54	0.18	3.71	0.26	2.17	0.27
Large Head Size	16.60	1.20	1.83	0.37	3.99	0.34	2.24	0.39

Table 3

Summary of Mean Air-Conduction Latency Findings for the Left Ear

Subject Group	Head Size		Wave I Latency, Right Ear		Wave V Latency, Right Ear		Wave I-V Interpeak Latency, Right ear	
	cm	*SD*	ms	*SD*	ms	*SD*	ms	*SD*
Small Head Size	9.91	1.40	1.50	0.14	3.80	0.15	2.30	0.17
Large Head Size	16.60	1.20	1.74	0.14	4.04	0.39	2.33	0.39

Table 4

Summary of Mean Air-Conduction Amplitude Findings

			Right Ear				Left Ear			
	Head Size		Wave I Amplitude		Wave V Amplitude		Wave I Amplitude		Wave V Amplitude	
	cm	*SD*	µV	*SD*	µV	*SD*	µV	*SD*	µV	*SD*
Small Head Size	9.91	1.40	0.;49	0.49	1.39	1.24	0.53	0.29	1.51	0.93
Large Head Size	16.60	1.20	0.34	0.13	0.84	0.34	0.41	0.09	0.92	0.39

Bone-conduction brainstem auditory evoked responses were obtained for each subject for analysis. Overall morphology of the waveforms was good with clear, identifiable Wave V peaks present and repeatable for all subjects. Sample waveforms are shown in Figure 3. A clear and identifiable Wave I was not present in all subject waveforms. All waveforms were obtained using alternating polarity; for no subjects was repetition using condensation polarity necessary. A minimum of 500 sweeps was obtained per waveform with a maximum of 1,000 sweeps. Once the two repeatable waveforms were recorded, they were averaged together in the software for analysis. On the resulting waveform, the absolute latency and peak-to-trough amplitude of Wave V was marked. Wave I latency and amplitude were recorded on waveforms where wave I was identifiable and repeatable. In such cases, the interpeak latency for Wave I-V was also recorded. A summary of the bone conduction waveform data can be found in Table 5.

The data are further summarized into mean head size, latency, and amplitude for Wave V for each test group in Table 6. Only 11 subjects had an identifiable wave I on their bone conduction waveform. Therefore, a Wave I-V interpeak latency could only be calculated for these 11 subjects. Mean Wave I-V latencies are summarized in Table 6.

Table 5

Summary of Bone-Conduction Brainstem Auditory Evoked Response Latencies and Amplitudes

Subject	Breed	Head Size (cm)	Wave I Latency (ms)	Wave I Amplitude (μV)	Wave V Latency (ms)	Wave V Amplitude (μV)	I-V Latency (ms)
1	Labrador Retriever	18.47	1.27	0.25	3.95	1.11	2.68
2	Boxer mix	17.71			3.75	1.39	
3	Siberian Husky	17.42			4.17	1.57	
4	Labrador Retriever	17.34	1.73	0.75	4.47	0.67	2.74
5	Shepherd mix	16.98			3.58	2.31	
6	Labrador Retriever	16.72	1.95	0.35	4.38	1.35	2.43
7	Olde English Bulldogge	16.02	1.77	0.36	3.98	0.77	2.20
8	Siberian Husky mix	15.25			3.92	1.73	
9	Berger Picard	15.09	1.80	0.36	3.98	1.42	2.18
10	Australian Cattle Dog	15.02	1.85	0.57	3.80	1.93	2.30
11	Cocker Spaniel mix	12.20			4.03	5.34	
12	Russell Terrier	12.02			3.80	1.67	
13	Fox Terrier mix	10.97	1.48	1.07	3.85	1.04	2.38
14	Chihuahua	10.16			3.77	2.20	
15	Miniature Rat Terrier	9.59	1.68	0.69	3.58	1.74	1.90
16	Yorkshire Terrier	9.46			3.60	2.93	
17	Chihuahua mix	9.04	1.93	0.31	3.92	3.30	2.00
18	Chihuahua	8.84	1.30	0.17	3.67	2.83	2.37
19	Miniature Rat Terrier	8.65			3.83	5.99	
20	Miniature Rat Terrier	8.20	1.80	0.61	3.83	2.69	2.03

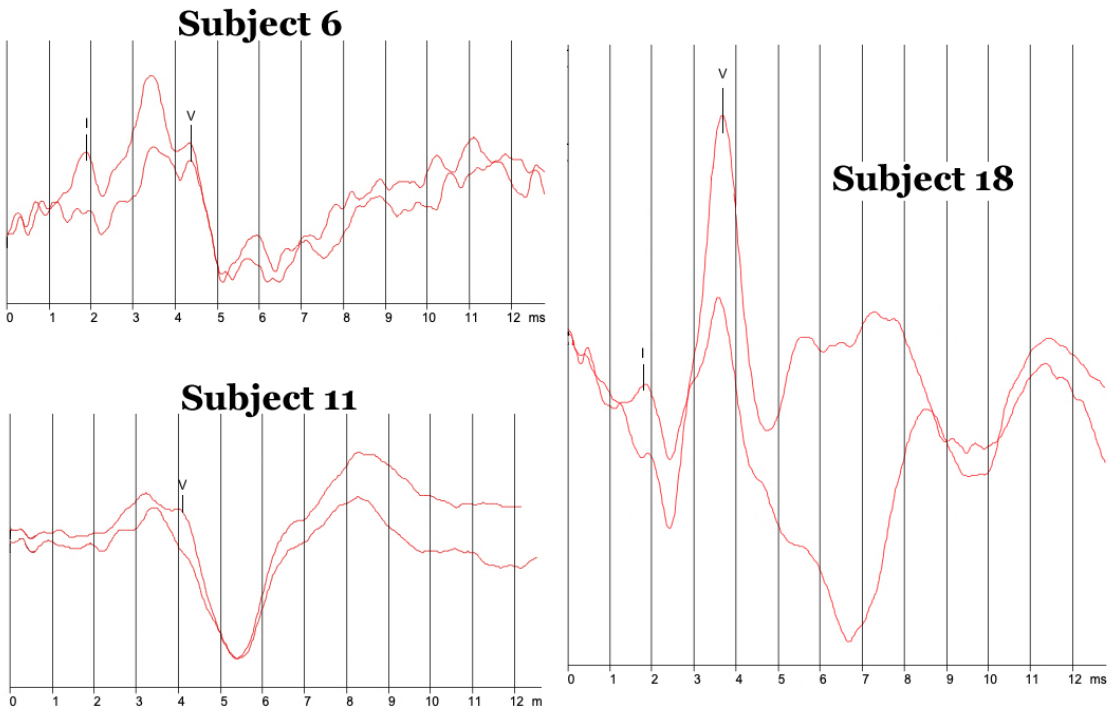
Table 6

Mean Head Size, Wave F Latency, Wave V Amplitude, and Wave I-V Interpeak Latency for Each Group and the Whole Sample for Bone-Conducted Brainstem Auditory Evoked Response

Subject Group	*n*	Mean Head Size		Wave V Latency		Wave V Amplitude		Wave I-V Interpeak Latency		
		cm	*SD*	ms	*SD*	µV	*SD*	ms	*SD*	*n*
Whole Sample	20	13.26	3.66	3.89	0.24	2.20	1.39	2.29	0.27	11
Small Head Size	10	9.91	1.40	3.79	0.14	2.97	1.58	2.14	0.22	5
Large Head Size	10	16.60	1.20	4.00	0.28	1.43	0.50	2.42	0.24	6

Figure 3

Bone-Conduction Brainstem Auditory Evoked Response waveform examples



Statistical analyses performed are summarized in Table 7, including *p*-value and Pearson's *r*. Among the data were two outliers in amplitude, subject 11 and subject 19, both occurred within the small head size group. Outliers were determined to be the top 5% of amplitude values, which were then excluded for comparative analysis. Statistical values for analyses performed while excluding the outliers are included in Table 7 in a separate line. Figure 4 illustrates the correlation between head size and Wave V latency. The correlation between head size and Wave V amplitude is shown in Figure 5.

Table 7

Summary of Mann-Whitney U Test and Pearson's r Findings for the Relationship Between Head Size and Wave V Latency and Amplitude

	n	Mann-Whitney *p*-value	Pearson's *r*
Head Size & Wave V Latency	20	0.0751	0.4929
Head Size & Wave V Amplitude	20	0.0065	-0.5789
Head Size & Wave V Amplitude (excluding outliers)	18	0.0209	-0.6944

Figure 4

Comparison of Head Size and Wave V Latency

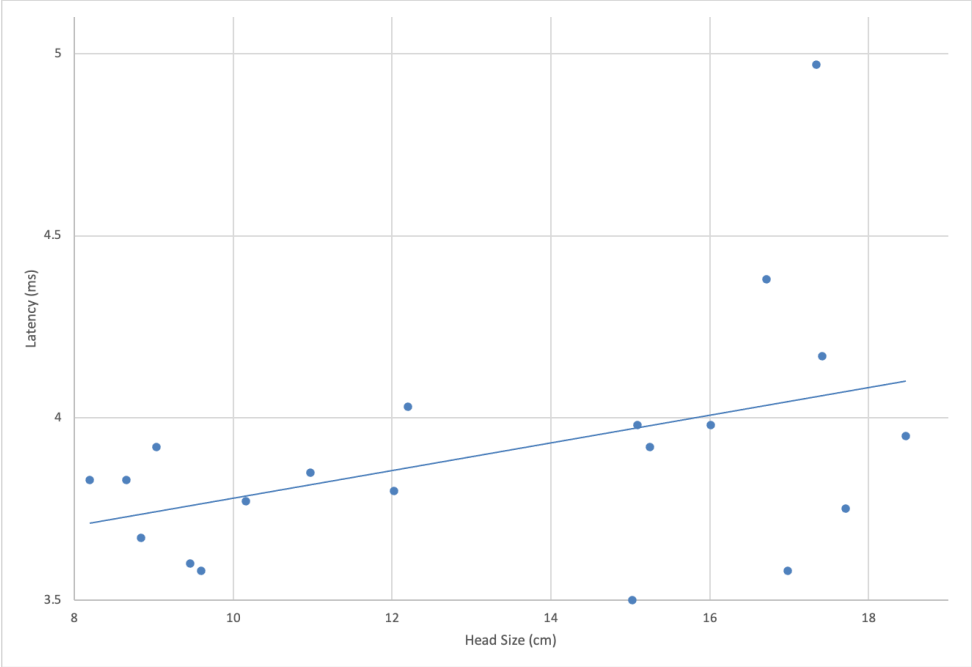
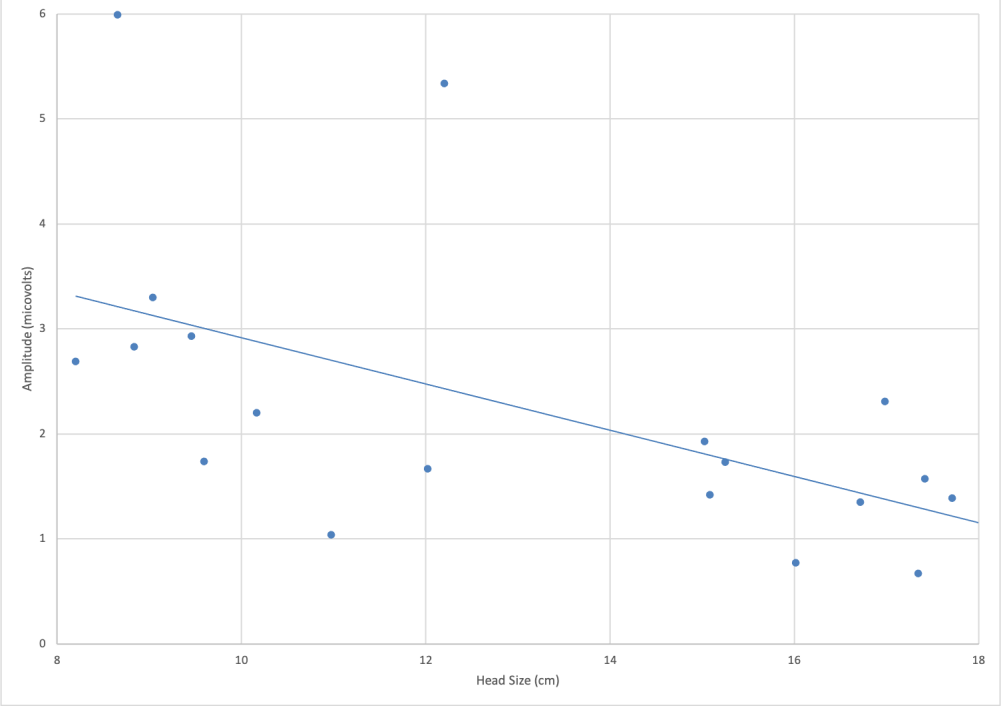


Figure 5

Comparison of Head Size and Wave V Amplitude



Pearson's *r* was also calculated to determine the relationship between age in months and Wave V latency as well as the relationship between age in months and Wave V amplitude. Both of these relationships are non-significant. This is summarized below in Table 8.

Table 8

Summary of Pearson's r Findings for the Relationship Between Age in Months and Wave V Latency and Amplitude

	n	Pearson's *r*
Age in months & Wave V Latency	20	0.0461
Age in months & Wave V Amplitude	20	-0.1449

CHAPTER V

DISCUSSION AND CONCLUSIONS

This study was designed to investigate the effects of measured head size on the latencies and amplitudes of Wave V in bone conduction BAER waveforms. Absolute latency of Wave V and the peak-to-trough amplitude of Wave V were analyzed for two groups: dogs with small head sizes, and dogs with large head sizes. Based on the findings of the current published literature, it was hypothesized that BAER waveforms recorded from dogs with larger heads would be characterized by later Wave V latencies than those recorded from dogs with smaller heads. Additionally, it was hypothesized that there would be no difference in amplitude of Wave V between the small and large groups.

Summary and Interpretation of Results

Clear waveforms with an identifiable and repeatable Wave V were obtained from all subjects when utilizing bone conduction stimuli. In comparing the absolute latencies for the small and large head size groups, a marginal difference was observed between groups. The mean Wave V latencies and standard deviations obtained from air-conducted stimuli and from bone-conducted stimuli were overall similar within groups. Mean bone-conducted Wave V latency for the small head group (mean head size of 9.91 ± 1.40 cm) was 3.79 ± 0.14 milliseconds while the mean air-conducted Wave V latency for the small head group was 3.71 ± 0.26 milliseconds in the right ear and 3.80 ± 0.15 milliseconds in the left ear. The mean bone-conducted Wave V latency for the large head group (mean head size of 16.60 ± 1.20 cm) was 4.00 ± 0.28

milliseconds, while the air-conducted Wave V latency was 3.99 ± 0.34 milliseconds in the right ear and 4.04 ± 0.39 milliseconds in the left ear. Bone-conducted Wave V latency means are reported in Table 6. A Mann-Whitney *U*-test was performed to determine if this difference between groups was statistically significant. This is a nonparametric test that is equivalent to an unpaired *t*-test and is used when sample sizes are small and no assumption can be made about the distribution. The *p*-value was reported as $p = 0.0751$, which is not considered statistically significant at $\alpha = 0.05$. This result is consistent with the findings of Kemper et al. (2013) when investigating the effects of canine head size on air conduction BAER waveforms. Furthermore, the results of this study are consistent with the findings of Munro, Paul, et al. (1997), who observed consistently shorter Wave V latencies in bone conduction BAER waveforms obtained from Jack Russell Terriers than in those recorded from Dalmatian subjects, with Jack Russell Terriers being smaller in size than Dalmatians. In both the case of Munro, Paul, et al. (1997) and Kemper et al. (2013), these latency differences were determined to not be statistically nor clinically significant. To further assess the data surrounding the relationship between head size and Wave V latency, correlation between measured head size and bone-conduction Wave V latency was assessed using Pearson's *r*. A relatively weak positive correlation ($r = 0.4929$) was found, suggesting that as head size increases, Wave V latency also increases.

Mean Wave V amplitude for the small head size group was 2.97 ± 1.58 μV and for the large head size group, 1.43 ± 0.50 μV. A Mann-Whitney *U*-test determined that this difference in amplitude between groups was statistically significant ($p = 0.0065$). Two outliers existed within the small head size group, one with a Wave V amplitude of 5.99 μV and one with an amplitude of 5.34 μV. It is suspected that these outliers may have occurred due to proximity of the bone oscillator to the subdermal electrode, which would likely produce a larger amplitude of Wave V.

Both outliers occurred within the small head size group where physical head dimensions and anatomy resulted in the zygomatic arch being in closer proximity to the electrode placed inferior-anterior to the tragus. To ensure that these outliers did not skew the statistical analyses, the Mann-Whitney *U*-test was re-run with these two data points excluded. Excluding these outliers revealed that the difference in Wave V amplitude between groups remained significant with $p = 0.0209$. Furthermore, Pearson's *r* was used to assess correlation between head size and Wave V amplitude. A negative correlation ($r = -0.5789$) was found when including all data points. When excluding the two aforementioned outliers to ensure that they did not unfairly skew the data, the strength of the negative correlation increased to $r = -0.6944$. This suggests amplitude of Wave V decreases as head size increases.

Strain (2011) noted that canine BAER waveforms can exhibit increased wave latencies and decreased amplitudes as the subject increases in age. These characteristics do not necessarily manifest in a noticeable hearing loss. To determine if this trend was also seen in the data collected, correlation coefficients for age in months and Wave V latency, and age in months and Wave V amplitude were calculated. The correlation between age in months and Wave V latency was very weak at $r = 0.0461$. Similarly, the correlation between age in months and Wave V amplitude was also weak at $r = -0.1449$. The two oldest subjects included in this study were 158 months and 152 months of age. The average bone conduction Wave V latency for these data was 3.89 milliseconds; the dog aged 158 months had a Wave V latency of 3.58 milliseconds, and the dog aged 152 months had a Wave V latency of 3.60 milliseconds. Both of these latencies fell below the mean for the whole sample. Therefore, the age-related effects described by Strain (2011) were not seen in the current study.

It remains unclear how or why head size affects both the latency and amplitude of Wave V. Determining such causations is beyond the scope of this study. However, the current literature proposes that the anatomical dimensions themselves could be responsible for these differences (Munro, Paul, et al., 1997). Logically, it could be suspected that larger anatomical dimensions would also result in larger dimensions of underlying structures, such as the brainstem and nerves along the auditory pathway. Considering the Wave I-V interpeak latencies, the large head size group had a mean of 2.42 milliseconds (±0.24 ms) whereas the small head size group had a mean Wave I-V interpeak latency of 2.14 milliseconds (±0.22 ms). Furthermore, larger dimensions between anatomical structures would understandably result in longer transmission times of neurological signals (Munro, Shiu, et al., 1997). This would occur simply as a result of the neurological signal having to travel longer distances from the cochlea, where the electrical signal is initiated, to the lateral lemniscus, where Wave V is believed to be generated (Møller, 2013).

In exploring possible explanations for differences in Wave V amplitude between the two head size groups, near-field and far-field recordings should be considered. Near-field potentials are recorded directly from or in extremely close proximity to the source of the electrical activity (Atcherson & Stoody, 2012). Far-field potentials are those that are recorded at larger distances from the source. It has been well established that potentials recorded closer to the source exhibit higher voltage. In other words, as the location of the recording increases in distance from the source of the potential, the voltage of the activity decreases (Atcherson & Stoody, 2012). While the BAER is a far-field evoked auditory potential recorded via subdermal electrodes on the scalp, it would be reasonable to consider the smaller anatomical proportions of the smaller head size group in interpreting the Wave V amplitude. As anatomical dimensions were considered in interpreting the decreased Wave V latencies in the small group, the same smaller anatomical

dimensions, particularly those of the skull and underlying structures, could also result in recordings of higher voltage. In a study by Plantz et al. (1974), the effects of electrode placement on the recorded magnitude of far-field evoked potentials was examined in rats. It was concluded that due to the small head size, recording auditory evoked potentials on the scalp of a rat cannot be considered to truly be far-field, as demonstrated by significant waveform changes produced by minimal movement of the recording electrodes. Measuring such a far-field potential on a small head results in the potential becoming closer to a near-field potential. While the dogs within the small head group are considerably larger than rats, this suggestion by Plantz et al. serves as an extreme example of the influence of head size on how "near" a far-field recording can become. As such, while the BAER recorded potentials are considered far-field potentials for both the small and large groups, due to the smaller structures and dimensions of the small group, it is reasonable to hypothesize that the recording electrodes are closer to the source in the small group than in the large group, thus resulting in larger Wave V amplitudes as measured in microvolts.

Strengths, Limitations, and Future Research

The most significant limitation to this study was the small sample size (n = 20). Conclusive, generalized statements about the relationship of canine head size on bone conduction BAER waveforms cannot be made without replicating the findings in a future study with a considerably larger sample to further validate the results. Furthermore, the dogs used in this study had a notable range of head sizes within each group; the difference in head size between the largest "small" headed dog and the smallest "large" headed dog was 2.82 cm. Future researchers may want to consider organizing the small and large head size groups to include dogs with a smaller range of head sizes within them to ensure a more consistent head size within

groups. Limited availability of dogs for this study within the time constraints of data collection resulted in the inclusion of a wider head size range than ideal.

All dog subjects were well behaved and agreeable for the duration of testing. However, some recordings included an excessive amount of artifact during bone-conduction testing that may or may not have influenced the overall waveform morphology and repeatability. Factors that could contribute to producing artifact were not always readily identifiable; it is unclear if the artifact was from external factors or equipment function. Whenever possible, such recordings with a significant amount of artifact were discarded and repeated. Another consideration is the potential variability in force applied by hand to the bone oscillator. The amount of pressure applied by hand was the maximum amount of pressure that the subject would allow or tolerate, which is consistent with the method described in Munro, Paul, et al. (1997). However, it would be reasonable to suspect that the tolerance level varied between subjects, some dogs permitting more pressure to be applied than others. Furthermore, consistent application of pressure may have been interrupted during recording as the dog moved his or her head slightly. Subjects were comfortably restrained, and the head stabilized as much as possible, but as the subjects were not chemically sedated, some head movement was inevitable. This may have also contributed to the outliers within the small head size group when looking at Wave V amplitude. Slight movements of the head and therefore bone oscillator placement may have resulted in the oscillator shifting closer to the subdermal electrode, producing a higher amplitude, similar to what is described by Plantz et al. (1974).

Additional research investing the effect of head size on bone conduction BAER waveforms in dogs is needed to further understand the relationship. A future study that investigates electrode placement, similar to what was done in rats in the Plantz et al. (1974)

study, may offer additional insight. As mentioned, future studies should include small and large head size groups with smaller ranges of head sizes within them, and therefore a larger difference in head size between the two groups. Furthermore, a positive correlation (r = 0.4929) was found between head size and Wave V latency, and a negative correlation (r = -0.5789) was found between head size and Wave V amplitude.

Conclusions

In summary, bone conduction BAER waveforms were able to be obtained in numerous dogs of various head sizes with good reliability and with no chemical restraint. A total of 20 dogs were organized into two groups: 10 dogs in a small head size (8.20 cm - 12.20 cm) group and 10 dogs in a large head size (15.02 cm - 18.47 cm) group. Results indicate that there is no significant difference between the two groups when comparing the absolute latency of Wave V on a bone conduction BAER waveform. The data suggest that there was a significant difference between the two groups when comparing the amplitude of Wave V on the bone-conducted waveforms. Further studies should be performed to replicate and validate these findings, ideally with a larger sample size with more consistent head sizes within each group.

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APPENDIX A

INSTITUTIONAL ANIMAL CARE AND USE COMMITEE (IACUC) APPROVAL

UNIVERSITY OF NORTHERN COLORADO

IACUC Memorandum

To: Jennifer Weber

From: Laura Martin, Director of Compliance and Operations, ARF

CC: Amanda Stone, IACUC Files

Date: August 5, 2019

Re: IACUC Protocol 1913C-JW-D-22 Approval

The UNC IACUC has completed a final review of your protocol "The Effect of Head Size on Bone Conduction Brainstem Auditory Evoked Response in Canines". The protocol review was based on the requirements of Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training; the Public Health Policy on Humane Care and Use of Laboratory Animals; and the USDA Animal Welfare Act and Regulations. Based on the review, the IACUC has determined that all review criteria have been adequately addressed. The PI/PD is approved to perform the experiments or procedures as described in the identified protocol as submitted to the Committee. This protocol has been assigned the following number 1913C-JW-D-22.

The next annual review will be due before August 5, 2020.

Sincerely,

Laura Martin, Director of Compliance and Operations

APPENDIX B

VETERINARY WELLNESS CHECK EXAMPLE FORM

FULL CIRCLE VETERINARY CARE
7 Rutherford, Johnstown, CO 80534
Phone (970) 587-5140

Client: _____
Phone: ( ) _____ - _____
( ) _____ - _____

PATIENT: _____ BREED: _____
COLOR: _____
DATE 11/15/19

HISTORY: No health concerns

1. Coat & Skin
☐ Appears Normal ☐ Itchy ☐ Bacterial Infection ☐ Flea (at Mo. S)
☐ Dull / Dry ☐ Shedding ☐ Fleas (at Mo. S) ☐ Hair Loss
☐ Scabs ☐ Matted ☐ Pigment
☐ Hotspot ☐ Tumors

2. Eyes
☐ Appears Normal ☐ Infection: L___ R___
☐ Discharge: L___ R___ ☐ Cataract: L___ R___
☐ Inflamed: L___ R___ ☐ Lenticular Sclerosis
☐ Eyelid Deformities ☐ Other _____

3. Ears
☐ Appears Normal ☐ Tumor: L___ R___
☐ Inflamed: ☐ Excessive Hair
☐ Itchy ☐ Yeast Infect: L___ R___
☐ Mites ☐ Bacterial Infect: L___ R___

4. Nose & Throat
☐ Appears Normal ☐ Inflamed Tonsils
☐ Nasal Discharge ☐ Enlarged Lymph Glands
☐ Inflamed Throat ☐ Other _____

5. Mouth, Teeth, Gums
☐ Appears Normal ☐ Gingivitis (Inflamed Gum Tissues)
☐ Broken Teeth ☐ Loose Teeth
☐ Tartar Buildup ☐ Pyorrhea (pus)
☐ Ulcers ☐ Tumors

6. Legs & Paws
☐ Appears Normal ☐ Nails Too Long
☐ Lameness(LF, RF, LR, RR) ☐ Joint Problems
☐ Damaged Ligaments ☐ Foot/Hair Discoloration

7. Heart
☐ Appears Normal ☐ Fast
☐ Murmur ☐ Other _____
☐ Slow

8. Abdomen
☐ Appears Normal ☐ Abnormal Mass
☐ Enlarged Organs ☐ Tense/ Painful
☐ Fluid ☐ Other _____

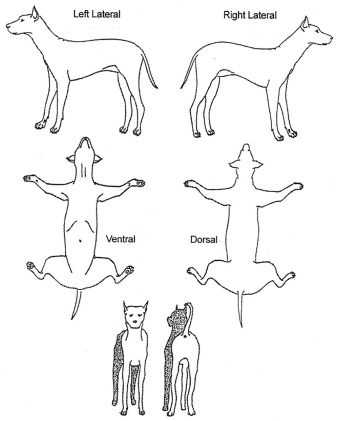
9. Lungs
☐ Appears Normal ☐ Breathing Difficulty
☐ Abnormal Sound ☐ Rapid Respiration
☐ Coughing ☐ Other _____
☐ Congestion

10. Gastrointestinal System
☐ Appears Normal ☐ Abnormal Feces
☐ Excessive Gas ☐ Parasites
☐ Vomiting Problem ☐ Other _____
☐ Anorexia (appetite)

11. Urogenital System
☐ Appears Normal ☐ Recommended Neutering
☐ Abnormal Urination ☐ Mammary Tumors
☐ Genital Discharge ☐ Anal Sacks
☐ Abnormal Testicles ☐ Enlarged Prostate

12. Weight 47.0 **Lbs.**
☐ Normal Range ☐ Thin by: _____ Lbs.
☐ Heavy by: _____ Lbs. ☐ Other _____

13. Diet
☐ Excellent ☐ Vitamin Needed
☐ Good ☐ Improvement Necessary



VITAL SIGNS: T 98.7 P = 150 R = 40

DESCRIBE ABNORMAL:

Rule Out:

RECOMMENDATIONS:
BAER testing w/ Fetch Lab
Limited Physical exam noted above

Vaccination Program ☐ Up to Date ☐ Vac.Due: ☐ Vac.Given:

DR. Mahoney Need _____ In _____ Days

Dogs
Annual Heartworm Test
☐ Negative
☐ Positive
☐ Recommended

Heartworm Refill?
☐ Yes
☐ No

Cats
Leukemia / Aids Test
☐ Negative
☐ Positive
☐ Recommended

Cats / Dogs
Annual Intestinal Worm Test
☐ Yes
☐ No
☐ Recommended
Result _____

Flea Control Needed
☐ Pet
☐ House
☐ Yard

Lab Results by
☐ Mail
☐ Phone
☐ Consult