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The Brainstem Auditory Evoked Response in Old Versus Young Horses

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THE BRAINSTEM AUDITORY EVOKED RESPONSE IN OLD VERSUS YOUNG HORSES

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ABSTRACT

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A brainstem auditory evoked response (BAER) is an objective test that measures changes in voltage in the ongoing electroencephalogram (EEG) in response to the presentation of acoustic stimuli, and is observed as a waveform with a known series of peaks. While the gold standard for measuring hearing sensitivity in humans is behavioral testing during which the listener provides a behavioral response to a sound (e.g., raising a hand or pressing a button), objective testing allows clinicians to estimate behavioral hearing sensitivity when behavioral testing is not possible. Therefore, BAER testing has been used as a tool to measure hearing in various animal species.

In humans, aging typically affects the brainstem auditory evoked response, producing waveforms with decreased amplitudes, wave V thresholds at higher stimulus intensities, and increased latency of wave V responses. The purpose of this study was to evaluate the effects of aging on the BAER in equines. The following research questions were investigated: Can brainstem auditory evoked responses be identified and replicated for older horses? If they can be identified, are there differences in response characteristics between young and old horses who have not participated in or been exposed to noisy situations in a convenience sample of horses? It was hypothesized that the older group of

horses would exhibit poorer thresholds, poorer morphology, increased latencies, and a decrease in amplitude of waves compared to the younger group.

Data were collected and analyzed from ten test subjects, five old (≥20 years) and four young (≤7 years) horses. Data obtained from one of the older horses was not included in the data analysis due to excessive noise from tooth grinding that obscured the waveforms. BAER testing was performed and waveforms were identified and replicated. Peak amplitudes, peak latencies, and thresholds were descriptively and statistically analyzed. There were no statistically significant differences between the two test groups.

It is unclear why age-related differences were not observed. It is possible that what is considered age-related hearing loss (presbycusis) in humans, is the cumulative effect of noise, ototoxicity, and other environmental factors. It is also possible that the click stimulus did not elicit responses from the frequency range most sensitive to age-related changes in horses. Future research should explore using other stimuli, such as high-frequency tonebursts, to evaluate other frequency ranges, determine the feasibility of sedating subjects to reduce artifact, evaluate test-retest reliability of the brainstem auditory evoked response in horses, and include brainstem auditory evoked response testing on a group of horses with noise exposure to compare with the results from this study.

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CHAPTER I

STATEMENT OF THE PROBLEM

Hearing loss related to aging, also known as presbycusis, is a form of sensorineural hearing loss that presents as increased hearing thresholds and changes in auditory processing (Boettcher, 2002). Schuknecht and Gacek (1993) summarized degeneration of the cochlear stria vascularis as the primary cause of presbycusis. Multiple researchers have examined the effects of presbycusis on the auditory brainstem response of humans, and it has been concluded that both decreased amplitude and increased latencies are the result of age-related hearing loss (Beagley & Sheldrake, 1978; Jerger & Johnson, 1988; Jerger & Hall, 1980; Rowe, 1978)

Boettcher (2002) reported that the greatest reduction of waveform amplitude is typically seen in wave V due to reduction in neuronal synchronicity. Latencies are increased due to a loss of hair cells within the cochlea, which also causes a decrease of neuron synchronicity. In 1978, Rowe studied peak and interpeak latencies in 25 young and 25 older subjects using click stimuli. Rowe reported that all wave latencies increased for the older adults, but only wave I to III interpeak latencies increased.

Jerger and Johnson (1988) examined the interactions among age, gender, and degree of hearing loss. Among records of 412 subjects, they found that when effective click level decreased, latencies increased in males. Jerger and Hall (1980) evaluated the effects of age and sex on amplitude and latency of wave V of the auditory brainstem response. They reported that latencies increased as a function of age for males and

females and the average latencies in the older age group were 0.20 milliseconds (msecs) longer than those of the younger age group. When discussing amplitude, they reported a decrease in the older age group, and a decrease twice as great in males compared to female subjects.

Beagley and Sheldrake (1978) investigated whether aging affected latency across seven decade groups from 11 years to 80 years old. A minimal increase in latency for wave V was found as a function of age; shorter latencies were seen for females in most cases, and a decrease in amplitude with increasing age was reported. Two possibilities were proposed for the decrease in amplitude: 1) synchrony following click stimuli decreases with age, and 2) tissue impedance increases with age.

Behavioral signs have been previously used to suggest deafness in equines, but in order to determine the presence and degree of hearing loss, brainstem auditory evoked response testing can be used to evaluate the integrity of the auditory pathway. Wilson, Mills, and Dzulkarnain (2011) listed four reasons brainstem auditory evoked response testing is needed. First, the animal's history only identifies the risks to the auditory system and not the function of the system; second, behavioral tests are unreliable and subjective, and also require training; third, the Preyer reflex (startle reaction) only indicates whether a severe to profound hearing loss exists; and lastly, a horse can compensate for its hearing loss with use of other senses.

With the ability to identify a horse's hearing status, training and handling can be positively impacted. If a horse has a hearing loss, it may not be able to hear commands or sounds in its environment. The owner must understand the communication needs of the horse, and be able to communicate commands to a deaf or hard of hearing horse. If

owners and trainers understand the horse's hearing loss, they are then able to train and handle the deaf or hard of hearing horse in a manner that is safe to both the trainer and the horse. Breeding programs may also be impacted, and genetic hearing loss could be decreased.

Examination of the literature indicates that many equine studies are limited in the explanation of parameters used to collect brainstem auditory evoked potential waveforms, or that inappropriate parameters were used. Multiple studies used a reference of dB nHL, which is an inappropriate measure when testing equines because it is based on normative data for humans (Aleman, Holliday, Nieto, & Williams, 2014; Aleman, Puchalski, Williams, Kass, & Holliday, 2008; Marshall, 1985; Wilson et al., 2011). Mayhew and Washbourne (1990) used dB HHL [*sic*] as well as a masker, both of which would be questionable parameters for testing horses. Also, limited findings from the study were discussed. Rolf, Reed, Melnick, and Andrews (1987) reported a noisy recording environment when the data was collected, which could have led to poor data. Magdesian, Williams, Aleman, LeCouteur, and Madigan (2009) did not include parameters of the brainstem auditory evoked response test aside from electrode placement. Aleman and colleagues (2014) reported parameters including the click stimulus level and masking level, but did not report any other parameters. Lastly, Aleman and colleagues (2008) used white noise masking improperly (based on current audiologic protocol).

The purpose of this study was to examine the effects of aging on brainstem auditory evoked responses in equines that had not been exposed to noise. Specifically,

threshold, latency, amplitude, and morphology were analyzed for groups of young and old equines.

Q1 Can brainstem auditory evoked responses be identified and replicated for older horses?

Q2 If they can be identified, are there differences in responses between young and old horses who have not participated in or been exposed to noisy situations in a convenience sample of horses?

H1 In those with responses, the older group of horses will exhibit higher thresholds, poorer morphology, increased latencies, and decreased amplitudes of waves compared to the younger group.

CHAPTER II

REVIEW OF THE LITERATURE

Presbycusis

Age-related hearing loss, also referred to as presbycusis, is a common form of sensorineural hearing loss observed in humans. It is characterized by elevated hearing thresholds and changes in auditory processing (Boettcher, 2002). Schuknecht (1974) identified four different types of presbycusis by examining human temporal bones: sensory presbycusis, a reduction in the number of hair cells; neural presbycusis, the loss of afferent nerve fibers; metabolic presbycusis, the loss of blood supply to the cochlea; and mechanical presbycusis, a "stiffening" of the basilar membrane and organ of Corti. In 1993, Schuknecht and Gacek reported degeneration of the stria to be the main problem in the temporal bones of elderly humans, and sensory cell loss (reduced numbers of hair cells), as being the least important cause of hearing loss in humans, especially when contributing elements such as noise, genetic defects, and drug exposure were removed.

Auditory Brainstem Response Overview

The auditory brainstem response was first measured about 45 years ago as a component of auditory evoked potentials. Auditory evoked potentials are derived from electrical activity of the subcortical structures and primary cortical areas in response to an acoustic stimulus. The auditory brainstem response is a specific auditory evoked potential occurring within 10 msec after a brief acoustic stimulus is presented, and is observed as a

waveform that contains seven peaks labeled wave I through VII (Jewett & Williston, 1971; Møller & Jannetta, 1982). Typically wave I occurs 1.5 to 2 msec after the stimulus, and each wave occurs at around 1 msec intervals following wave I. For humans, it is fairly common for waves II, VI, and VII to be absent, as well as for waves IV and V to merge. The auditory brainstem response is generated by the auditory nerve and the brainstem, with higher wave numbers corresponding to a more central location along the pathway (Boettcher, 2002; Jewett & Williston, 1971). In the United States, positive polarity is typically plotted upward while negative polarity is plotted downward (Arnold, 2007).

Anatomy and Physiology of the Auditory Brainstem Response

Over the years there has been much speculation about where each wave from the auditory brainstem response (I, II, III, IV, V, and VI) originates. Initially, most of the research was performed on animals and humans with known lesions, therefore making it difficult to generalize to the normally functioning human auditory pathway. Initial ideas about the sites where each wave was generated were found to be incorrect; the anatomy of the auditory brainstem response is much more complex than initially proposed. Some waves are generated by multiple anatomical regions and one anatomical region can create multiple waves (Møller & Jannetta, 1982; Møller & Jannetta, 1985; Møller, Jho, Yokota, & Jannetta, 1995).

Wave I of the auditory brainstem response has been clearly identified as the result of activity in the distal portion of the eighth nerve; specifically it is the result of synchronous firing of afferent nerve fibers as they leave the cochlea and enter the internal auditory canal. This has been illustrated by researchers who examined direct recordings

from the eighth nerve potentials. The negative trough that follows wave I also originates from the eighth nerve as it exits the internal auditory canal (Hashimoto, Ishiyama, Yoshimoto, & Nemoto, 1981; Møller & Jannetta, 1982; Møller et al., 1995).

Intracranial recordings have suggested that wave II is also a result of activity from the eighth nerve, stemming from the proximal portion of the nerve. In young children, wave II of the auditory brainstem response is often not recorded; this is hypothesized to be due to the shorter length of the eighth nerve in children as compared to adults (Møller & Jannetta, 1982; Møller & Jannetta, 1985; Møller et al., 1995).

Originally, wave III was associated with the superior olivary complex (SOC) within the brainstem. This may be due to studies originally done in small animals including cats and guinea pigs, which have subsequently been shown to produce different results in comparison to humans. Møller and colleagues found an association between the cochlear nucleus, ipsilateral to the stimulated ear, and the recorded wave III of the auditory brainstem response. Based on consensus from multiple studies, it was theorized that wave III is generated from, or near, the cochlear nucleus and the following trough results from the trapezoid body (Møller & Jannetta, 1982; Møller et al., 1995).

It has been speculated that wave IV originated from the lateral lemniscus, but due to research on humans resulting in different conclusions, the hypothesis was not widely adopted. Wave IV often appears close to, or leaning on wave V, often called the wave IV/V complex. Identifying the specific location where wave IV is produced has proven to be extremely difficult likely due to the many decussations (crossings over midline) of auditory fibers beyond the cochlear nucleus. Wave IV has also been thought to originate from with the inferior colliculus (Møller & Jannetta, 1982; Møller & Jannetta, 1985).

Møller and colleagues (1995) suggested that wave IV is generated by the superior olivary complex in humans.

Wave V is the wave most referred to clinically (Hall, 2007). According to Møller et al. (1995) “the sharp portion of peak V reflects the activity in the lateral lemniscus that has not been interrupted in the superior olivary complex or the nucleus of the lateral lemniscus” (p. 602). Following wave V is a large trough, thought to be associated with dendritic potentials that lie within the inferior colliculus.

For later waves, including waves VI and VII, it is more difficult to pinpoint the generating location. There is no consensus in the literature for the source generators of these later waves. Stockard and Rossiter (1977) suggested that they originate from the medial geniculate body within the thalamus, Hashimoto et al. (1981) agreed with the thalamic origin, while a study by Møller and colleagues associated these waves with firing of neurons from the inferior colliculus.

Recording of the Auditory Brainstem Response

The auditory brainstem response of humans is recorded non-invasively with electrodes placed on certain locations of the head using the 10-20 orientation, called an electrode montage (Jasper, 1958). Different electrode arrangements can be chosen to optimize the presence of specific peaks. Electrodes come in various forms and use varies by preference (King & Sininger, 1992). Jewett and Williston (1971) studied the results of the auditory brainstem response using various types and montages of electrodes. These methods included an electrode at the vertex and each side of the head as well as a needle in the ear canal; EEG disc electrodes placed on the scalp with a ground electrode placed on the opposite lobe or the neck and a hypodermic needle placed subdermally in the

posterior ventral wall of the ear canal near the tympanic membrane; and a saline-bridge wick electrode placed several millimeters from the tympanic membrane. They found that placing a needle in the ear canal and electrodes at the vertex and at each side of the head allowed many more components along the brainstem pathway, or waves, to be clearly discerned. Results from the surface electrodes in comparison to the needles showed more artifact, but waves were still viewable.

Surface electrodes, due to their comfort and ease of use, are typically used with humans (King & Sininger, 1992). Electrodes connect to an electrode box containing a preamplifier via wires. Each electrode is connected to a specific jack of the electrode box depending on the electrode montage chosen. The electrode box is then connected to a computer with software that is able to record and store the auditory brainstem response (Arnold, 2007).

Multiple electrodes are used in order to record evoked potentials by a technique called differential recording, which measures the difference in electrical activity between two electrodes using a differential preamplifier (Arnold, 2007). It is recommended that one electrode be placed on the vertex (the top of the head), which enhances wave V due to flow of energy up the brainstem; this serves as the noninverting electrode. Another electrode is placed on the earlobe or mastoid (the inverting electrode), and a third electrode serving as the ground is placed on the other earlobe or mastoid, or on the forehead or nape of neck serving as the ground electrode (King & Sininger, 1992). Prior to electrode placement the skin must be cleaned of oils, dead skin, and other debri. Conducting cream is placed on each electrode before positioning on the three areas

mentioned above unless adhesive or needle electrodes are used; in those cases conducting cream is not necessary (Arnold, 2007).

Electrode impedance is checked prior to recording and should be less than or equal to 5 kiloOhms. The impedance is determined by the contact potential between the electrode and the skin. This impedance contributes to the noise from the electrode and the amount of artifact that is recorded. Low impedance usually means less electrical noise interference and artifact within a recording. Balanced impedance among all electrodes is important due to the role of the common-mode rejection ratio (CMRR). CMRR is a measure of the amount of cancellation of common noise between two electrodes while the evoked potential is unchanged (Burkard, Eggermont, & Don, 2007).

To reduce unwanted electrical noise, differential amplification, filtering, and signal averaging are used when obtaining the auditory brainstem response waveform (Arnold, 2007). Poor signal-to-noise ratios can lead to poor amplitude, morphology, and latency. This can lead to errors in peak identification of recorded waveforms (Wong & Bickford, 1980).

Filtering during recording allows some response frequencies to pass through while others are attenuated. Generally, two filters are used: a high-pass filter which allows frequencies to pass above the cutoff frequency and a low-pass filter which allows frequencies below the cutoff frequency to pass. These filters are set based on the spectral energy within the auditory evoked potential of interest. Regions containing energy outside the pass band are eliminated so that the signal-to-noise ratio is increased (Burkard et al., 2007). Jewett, Romano, and Williston (1970) discovered that by using filtering (bandpass filter 10 Hz to 2 kHz), unwanted electrical noise was effectively eliminated

and results could be clinically useful for detecting problems in brain function. Jewett and Williston (1971) showed that latency and morphology were affected by the boundary of the low-pass filter and suggested that the low-pass filter setting should be greater than 1,000 Hz. Spivak and Malinoff (1991) assessed the effects of using digital filtering on improving waveform morphology and therefore peak picking. Peak detection was performed by eight clinicians who had experience in labeling peaks; results showed that filtering improved morphology of waveforms and the reliability of peak identification across clinicians.

Signal averaging allows the evoked potential response to be differentiated from background electrical noise because it is synchronized or time locked to the stimulus when compared to the random noise picked up during recording. Multiple sweeps (or samples) are obtained to collect a waveform, which includes evoked responses and random noise; electrical energy that is random is reduced, thus enhancing the signal-to-noise ratio of the response (Wong & Bickford, 1980).

Because of the amount of electrical noise the electrodes pick up, including physiologic noise, it is best to have the patient in a relaxed state during recording to decrease the amount of muscle activity (Arnold, 2007). In a study by Jewett and Williston (1971), the recordings yielding the most distinct waves were collected from patients that were the most relaxed and adapted to the testing procedure. Wong and Bickford (1980) also reported that a relaxed subject improved the signal-to-noise ratio of a recording.

Specific time windows, or epochs, are used in recording; these start when the stimulus is presented and end after all responses of interest are obtained. The time window used will determine which responses will be viewable. The typical time window

for adult subjects is 10 to 12 msec. For children, a longer time window of 20 to 25 msec is recommended because of the possibility of a lack of neural maturation which delays the latencies of waves. If the time window is too short, responses could be missed (Crumley, 2007).

Stimulus Parameters

The auditory brainstem response is elicited by a stimulus that has a rapid onset in order to synchronize the firing of neurons. Both click and toneburst stimuli are used to evoke the response (Stapells & Oates, 1997). A rapid click response, which is typically measured clinically, produces a waveform that reflects activity primarily from 1,000 Hz to 4,000 Hz (Hall, 2007). A click is a broadband stimulus, so low frequency regions of the basilar membrane respond as well, but are not recorded because the response from the high frequencies is faster. This is due to the traveling wave in the cochlea not being as effective for low frequencies as high frequencies when it comes to generating synchronous firing of eighth nerve afferent fibers (Stapells & Oates, 1997).

When selecting stimulus parameters for measuring an auditory brainstem response, both the frequency specificity and the duration of the stimulus should be considered. When brief stimuli are used, synchronous firing of neurons occurs; however, a larger frequency range is assessed due to spectral splatter. Stimuli that are longer in duration are more frequency specific but because the onset times of such stimuli are longer, they do not elicit a clear neural response. Pure-tone stimuli cannot be used due to the long onset of the tone; neurons would not fire in a synchronous manner and a response could not be recorded (Stapells & Oates, 1997).

Tonebursts are brief tones with short rise-fall times (Arnold, 2007). More frequency specific information can be obtained with the use of toneburst signals compared to clicks. This is essential for management of individuals with hearing impairment, including obtaining information prior to hearing aid fittings, and for assessing auditory sensitivity at different frequencies for newborn hearing screenings (Hall, 2007). The toneburst has energy centered around a single frequency, which activates the basilar membrane in the cochlea where the frequency-specific neural units lie. A toneburst that has a brief onset can produce "spectral splatter," the presence of unwanted frequencies. To overcome splatter, many methods have been devised including masking of either the stimulus or the tones produced above and below the intended frequency (Stapells & Oates, 1997). Jewett and Williston (1971) demonstrated that click stimuli were more effective than tonebursts for obtaining brainstem responses, but when tonebursts were set to record high frequency responses, results were similar between the two.

Stimulus rate is important for response clarity and test efficiency (Arnold, 2007). A slow stimulus rate produces a very clear waveform but increases the amount of time needed to signal average, while a fast stimulus rate reduces test time but decreases the amplitude of the waveform (Hecox & Galambos, 1974). In a study by Elberling and Don (1986), a decrease in amplitude, specifically for wave V, was seen when stimulus rate was increased above 30 clicks per second. Rates of 17 to 20 clicks per second are typically used clinically to ensure good waveform morphology (Arnold, 2007). These rates differ depending on the purpose of the testing.

Polarity of the stimulus is also a setting to consider when obtaining an auditory brainstem response. Options are rarefaction, condensation, or alternating rarefaction and condensation. For humans, rarefaction clicks have been shown to produce the clearest response in most cases (Hall, 2007).

Clinical Uses of the Auditory Brainstem Response

The following discussion applies to use of the brainstem response for evaluating human subjects. The brainstem response is typically used to assess two types of clinical cases.

Threshold Auditory Brainstem Response

Auditory brainstem responses are used to estimate auditory threshold for those who cannot give behavioral responses (Ackley, Herkzberger-Kimball, Burns, & Balew, 2006). Children are easily tested this way, as well as those who cannot respond with conventional testing (infants, individuals with developmental delay, individuals with autism etc.) Waveforms are consistent among people and the patient's state of arousal does not affect testing, which are reasons why this type of testing has become so popular and why it is so useful with infants (Arnold, 2007).

Click stimuli are presented first, collecting information between 1 kHz and 4 kHz followed by tonebursts to obtain more frequency-specific information if needed (Hall, 2007). A high intensity click stimulus is presented first in order to obtain a clear, robust waveform. Wave V is identified and reviewed at decreasing levels of click intensity to determine an individual's threshold (Guideline 9C: Guidelines on short-latency auditory evoked potentials, 2006). Wave V is evaluated because it is the most robust of the peaks and can be viewed at low intensity levels (Jewett & Williston, 1971). The lowest click

level at which wave V can be elicited gives an idea of an individual's hearing ability (Arnold, 2007). This threshold, or lowest click level in which wave V is visible is usually within 10 to 20 dB of the behavioral threshold within the 1 to 4 kHz frequency range (American Speech-Language-Hearing Association, 2004; Burkard et al., 2007).

Neurodiagnostic Auditory Brainstem Response

In addition to threshold estimation, the auditory brainstem response can be used as a neurodiagnostic tool. The neurodiagnostic auditory assessment detects lesions that may exist along the auditory pathway (Selters & Brackmann, 1977). Protocol for a neurodiagnostic auditory brainstem response includes a click stimulus presented at a slow rate at a high intensity level. Interpretation includes analyzing absolute latencies of waves I, III, and V, and interpeak latencies for wave I-III, III-V, and I-V. Amplitudes for waves I and V are also analyzed. Abnormal results are indicated when response latencies are greater than two standard deviations from the mean of an age-matched control. The area of the auditory brainstem response waveform that is affected may be indicative of where the lesion lies on the auditory pathway. The auditory brainstem response has been reported to have a 98% sensitivity rate for diagnosing large tumors of the auditory nerve and brainstem (Ackley et al., 2006).

Effects of Aging on the Auditory Brainstem Response

Presbycusis can significantly influence auditory brainstem response results, therefore influencing interpretation of responses for older individuals in comparison to younger individuals. Typically, there is a relationship, or correlation, between behavioral and auditory brainstem response thresholds however, this is not consistently the case for individuals with presbycusis. This is likely due to a reduced number of spiral ganglion

fibers and reduced synchrony of elements involved in producing the auditory brainstem response, which affect neural transmission but not necessarily hearing threshold; therefore, the auditory brainstem response may overestimate the behavioral hearing threshold for an older individual. A decrease in auditory brainstem response amplitudes is typically seen; the most reduction is seen in wave I resulting from a reduction in neurons, a reduction in synchronicity, and a reduction in the evoked potential. Absolute latencies tend to increase, but few studies have noted increases regarding interpeak latencies. Latencies are affected due to loss of hair cells located at the point of maximum displacement, as well as a decrease of neuron synchronicity (Boettcher, 2002).

In 1978 Rowe studied peak and interpeak latencies in 25 young (17 to 33 years) and 25 older (51 to 74 years) subjects using click stimuli. At 60 dB using 10 clicks/second, young subjects' peak latencies were as follows: wave I 1.87 msec, wave II 2.88 msec, wave III 3.83 msec, wave IV 5.06 msec, and wave V 5.82 msec. For the older subject group peak latencies were as follows: wave I 2.17 msec, wave II 3.36 msec, wave III 4.35 msec, wave IV 5.43 msec, wave V 6.16 msec. For the older age group the interpeak intervals for waves I-III and III-V were 2.19 and 1.82, respectively. Rowe reported that all wave latencies increased for the older adults, and I-III interpeak latencies increased while III-V latencies did not.

Jerger and Hall (1980) studied the effects of age and sex on amplitude and latency of wave V of the auditory brainstem response. They found that latency increased as a function of age for males and females; the average latency increased by 0.20 msec over the age range from 25 years to 55 years. When combining all age groups, male subjects' latencies were on average 0.14 msec greater than those for female subjects. When

looking at amplitude, a decrease was found for the older age group, and a decrease twice as great was seen for the males. Jerger and Johnson (1988) examined the interactions among age, gender, and degree of hearing loss. Among records of 412 subjects, they found that when effective click level decreased, which was calculated according to the difference between the intensity of the click that was used to elicit a response and a subject's hearing threshold level at 4 kHz, latencies increased for males but not for females. The gender difference was considered to be related to hormonal differences.

Beagley and Sheldrake (1978) investigated whether aging affected latency across seven decade groups from 11 years to 80 years with 10 subjects within each subject group; groups were split evenly male to female. A minimal increase in latency for wave V was seen as a function of age. Shorter latencies were seen in females in most cases, as was reported in previous studies. A decrease in amplitude in relation to age was also found. Because wave V is the most consistent and visible of the waves, amplitude was measured by assessing the amplitude of wave V from crest to trough. Two possibilities were offered to explain the decrease in amplitude: neural synchrony becomes poor with advancing age, and there is increased tissue impedance with age.

Ottaviani, Maurizi, D'Alatri, and Almadori (1991) evaluated 74 subjects affected by presbycusis for retrocochlear involvement. For each subject, the Metz's profile was used to look at presence of recruitment, the absolute latency of waves I, III, and V were assessed, and the interpeak latencies between waves I-III, III-V, and I-V were calculated. Results indicated an increase in latency in those individuals with presbycusis, and the increase in latency was linked with the shape of the hearing loss, especially within the 1 to 4 kHz region. A difference in interpeak latencies was not seen.

In a study by Konrad-Martin et al. (2012), auditory brainstem responses were recorded from 131 male veterans between the ages of 26 and 71. Amplitude, latency, and interpeak intervals (IPI) were examined for each individual. With regard to auditory brainstem response amplitudes, aging reduced the amplitudes of each peak. Multiple click rates were tested when evaluating IPIs and changes associated with aging were not seen. Statistically significant latency shifts were noted at waves I and III but not across any other waves; wave I increased by 0.25 msec (16%) and wave III increased by 0.29 msec (8%) with age. The researchers theorized that aging causes a reduction in the amount and/or synchrony of firing from the contributing units in the auditory nerve and cochlear nucleus, but it is more likely a reduction in numbers of contributing units rather than synchrony.

Brainstem Auditory Evoked Response Testing in Canines

Clinical brainstem auditory evoked response testing of animals has primarily been used for evaluating canine hearing. The test is the same as the auditory brainstem response conducted in humans, but it is typically referred to as a brainstem auditory evoked response test, or BAER, when testing animals. It is effective for screening breeds that have high risk for congenital deafness, which has been reported among more than 90 breeds (Strain, 2011). It is also important to determine that working and service dogs have normal hearing to ensure the ability to hear commands given to them. Furthermore, diagnostic testing is needed to determine presence of presbycusis, to identify ear pathologies, and to identify acquired and progressive hearing losses due to noise exposure (Scheifele & Clark, 2012).

Because of the objective nature of the brainstem auditory evoked response, it is more accurate than observation of the Preyer reflex, which is the movement of the ears that occurs in some animals in response to a suprathreshold noise. This type of behavioral testing is not sufficient for determining threshold levels, and cannot identify unilateral losses. Assessment of the brainstem auditory evoked response in the dog depends on the comparison of that dog's response to normative data. There is, however, lack of agreement about a universal test protocol making current data unable to be compared and duplicated. Scheifele and Clark (2012) recommend specific test protocol settings for canine evaluation.

The computer-based equipment used for obtaining canine brainstem auditory evoked responses is no different than the equipment used to test humans and can be separated into recording and stimulus components. Among the recording components are recording electrodes, display screen, differential amplifier, and a signal averager. The stimulus components consist of a stimulus generator and a transducer. Recommendations by Schiefele and Clark for settings are as follows. For the amplifier, an absolute gain setting of 100,000 to 150,000 is typical, and is set to record in microvolts. Regarding filters, a high-pass cutoff filter should be set at 300 Hz, and the low-pass filter at 1.5 kHz. The signal averager should run 1,000 to 2,000 sweeps for each stimulus level. The stimulus type used is a 100 microsecond broadband click with 12,000 Hz bandwidth power. Stimulus polarity using rarefaction clicks in humans has shown to generate the clearest response, while for canines, no research has been produced to determine the best stimulus polarity. A stimulus presentation rate of 33.3 clicks per second has been found to produce the best waveform results in the least amount of time. Stimulus intensity

recommendations include using dB peSPL units as a standardized method for reviewing results. “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). For diagnostic tests, five stimulus intensities are recommended: 70 dB peSPL, 80 dB peSPL, 90 dB peSPL, 102 dB peSPL, and 116 dB peSPL. Lastly, an insert earphone transducer is best to reduce stimulus artifact, provide the most comfort, prevent collapsing of canals, and reduce background noise (Scheifele & Clark, 2012).

If sedation is not used, a topical anesthetic cream, 2.5% lidocaine and 2.5% prilocaine cream, can be applied to electrode placement areas to prevent pain or discomfort. Subdermal electrodes with a diameter of 13mm are placed at three locations; this placement is called the electrical montage. The positive, non-inverting electrode is placed on the vertex (Cz), or midline of the head. The negative, or inverting, electrode is placed just in front of the tragus (A1) of the ear being tested, and the non-test ear receives the ground electrode (A2) with the same placement as the inverting electrode, just forward of the tragus. Subdermal electrodes are best for quick, secure, and consistent placement. The electrodes are then attached to a preamplifier electrode box. Prior to each recording, the impedance of the electrodes should be tested; the impedance should be relatively equal among the electrodes and should be 5 kiloOhms or less for each electrode.

The configuration of electrodes mentioned above has been found to be the best for recording the auditory nerve and brainstem components while also being the least impacted by electrical noise. A minimum of two waveforms at each intensity level are

acquired to determine reproducibility of the response. When estimating hearing threshold in humans, identification of the lowest intensity in which a wave V is present suggests threshold would be within 10 to 20 dB of that level. This type of estimation is used for the canine population as well (Shiefele & Clark, 2012).

Presbycusis in Canines

Shimada, Ebisu, Morita, Takeuchi, and Umemura (1998) researched the morphological changes of the cochlea and the cochlear nuclei related to presbycusis in canines. A group of 23 dogs was assessed, ranging in age from 3 days to 17 years. Similar changes that are viewed in humans were also seen in dogs; these included loss of spiral ganglion cells, atrophy of the organ of Corti and the stria vascularis, and thickening of the basilar membrane. All of these changes were more severe at the base of the cochlea than the apex. All changes seemed to progress as a function of age, and all four types of changes were found in dogs over 12 years old. Changes in cochlear nuclei included nerve cell loss and an abnormal increase in the number of astrocytes and ubiquitin removal; these changes were found in all dogs over 10 years old. These morphological changes viewed within the cochlea and the cochlear nuclei were, but not always, in conjunction with hearing loss.

In 2008, ter Haar, Venker-can Haagen, van den Brom, van Sluijs, and Smoorenburg evaluated whether the hearing of dogs becomes impaired with increased age as it does in humans. They suspected that canine hearing would become impaired across the entire frequency spectrum, but as observed in humans, would primarily be concentrated in the high frequencies. Three groups of 10 dogs were assessed. Group I had a mean age of 1.9 years, group II had a mean age of 5.7 years, and group III had a mean

age of 12.7 years. Results showed that group III had significantly higher thresholds than groups I and II across all frequencies. Group II showed an increase in thresholds associated with age, beginning around 8 to 10 years and exhibited the greatest impact in the 8 to 32 kHz frequency range.

While the research base regarding brainstem auditory evoked response testing in canines is growing, research is limited on testing of equines. Just as in canines, it is important to determine hearing status in horses for training purposes as well as breeding purposes. It can also be helpful in determining presence of ear pathologies, presbycusis, and noise-induced hearing loss. Thus far, consistent parameters have not been identified and a gold standard is needed.

Anatomy and Physiology of the Equine Hearing System

Blanke, Aupperle, Seeger, Kubick, and Schusser (2014) looked at the histological composition of the external, middle, and inner ear structures in horses and how they compare to humans. Many anatomic similarities were found between horses and other mammals including those of the pinna, external auditory meatus, tympanic membrane, ossicles, the cochlea and associated structures, as well as the vestibular system. Differences that were noted include ridges or circular wrinkles in the external auditory meatus that are visible in an otoscopic evaluation. Absence of these rings is indicative of swelling or increased humidity within the outer ear. The tensor tympani muscle, located in the middle ear cavity, is very prominent in the horse, with a fan-shaped appearance of many muscle fibers embedded within the adipose tissue. The number of turns in the cochlea varies across mammals; the equine cochlea has 2.25 turns, rather than the 2.5 to 2.75 turns humans possess. Overall, these differences and similarities are important to

consider when making comparisons to test results from other mammals. For example, a short basilar membrane may result in shorter latencies of waves (Blanke et al., 2014).

Behavioral Evaluation of Hearing in Equines

Heffner and Heffner (1983) looked at the auditory sensitivity of the horse using a "go/no-go" behavioral procedure. This type of procedure occurs when an animal is rewarded for making a specific response, in this case when a horse hears a sound. A thirsty horse was led into a stall and trained to place its nose on a plate in front of a loudspeaker. When the horse heard the tone it was trained to move from the observing plate and touch a reporting plate within 3 seconds of the sound being elicited. When the horse completed the task correctly it was rewarded with water.

Tonal sounds were presented and intensity decreased, and threshold was taken at the lowest level where the horse responded 50 percent of the time. An audiogram was then constructed representing the thresholds obtained. Sensitivity improved at 500 Hz and was consistent until around 33.5 kHz. In comparison to humans, the low frequency responses for horses were somewhat worse. Specifically, for speech frequencies (500 Hz to 8 kHz), humans had better thresholds than horses. As frequency increased beyond 8 kHz it was apparent that equine sensitivity was better than that of humans. The best sensitivity was within the range of 1 kHz to 16 kHz with a worsened sensitivity at 4 kHz. At around 16 kHz, sensitivity began decreasing rapidly. Figure 1 illustrates the audiogram recorded from threshold testing (Heffner & Heffner, 1983).

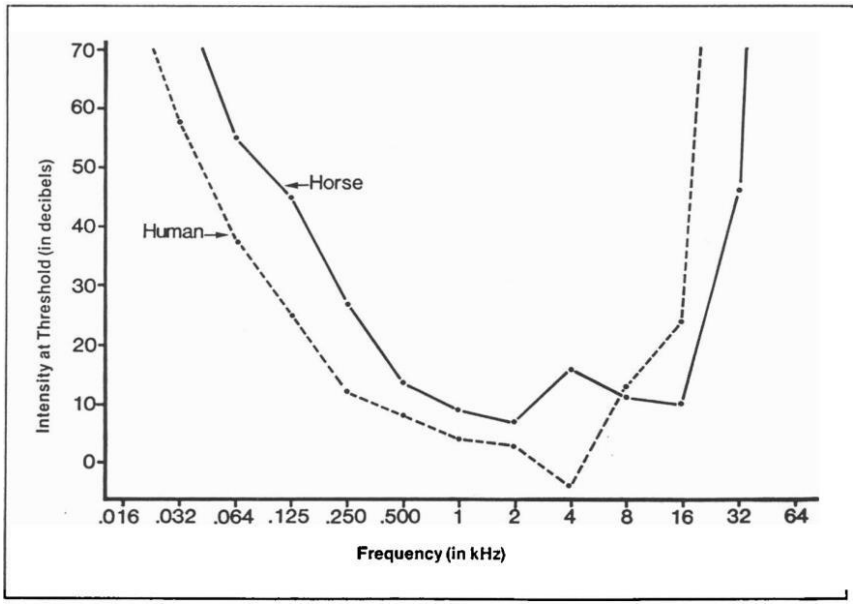

Figure 1. Horse audiogram. Reprinted from "The hearing ability of horses," Heffner & Heffner, 1983, *Equine Practice*, 5, p. 30. Copyright 1983 by Henry Heffner. Reprinted with permission.

Odberg (1978) looked at horses' behavioral responses to several high frequencies using a Galton whistle. Ten horses were tested: 2 thoroughbreds and 8 half-blood horses. Of these, 5 were mares with ages of 5, 6, 6, 7, and 18, and 5 were geldings ages 7, 9, 9, 15, and 18. A low-frequency Hewlett-Packard generator was used in combination with an RCA amplifier and a Stephens Trusonic tweeter to amplify frequencies ranging from 14 kHz to 25 kHz. Each frequency was tested 10 times for a total of 120 presentations in random order. If the horse appeared inattentive, a frequency was not presented (Odberg, 1978).

Two response reactions were recorded: (1) the Preyer reflex, twitching of the pinna; and (2) a fright reaction (the animal jumped away). Each horse was tested in the same area within 3 meters of the sound instrument through a hole in a cloth screen that was used to hide the equipment. Of reactions recorded, most were the Preyer reflex, and only a few elicited a fright reaction. Each horse heard all frequencies, but a decline in

reactions was found as frequencies rose from 14 kHz to 24 kHz, then an increase in reactions when the frequency reached 25 kHz. The author noted that as frequency increased there was a decrease in intensity, which could have caused the decrease in response. There was a significant difference in the number of responses between the younger and older horses, "older" consisting of those 15 and 18 years old. The authors concluded that this shows that the horse's auditory function decreases with age (Odberg, 1978).

Brainstem Auditory Evoked Response Testing of Equines

Behavioral signs have been previously used to suggest deafness, but in order to determine the presence and degree of hearing loss, brainstem auditory evoked response testing can be used in horses to evaluate the integrity of the auditory pathway. Wilson and colleagues (2011) listed four reasons needed for brainstem auditory evoked response testing. First, the animal's history identifies only the risks to their auditory system; second, behavioral tests are unreliable and subjective, and also require training; third, the Preyer reflex (startle reaction) only tells whether a profound to severe loss exists; and lastly, a horse can compensate for its loss with use of other senses. Identifying a horse with a hearing loss could prevent risk of injury in noisy environments for both the horse and the owner (Wilson et al., 2011).

Studies Prior to 2008

Marshall (1985) evaluated 10 horses and 7 adult ponies using brainstem auditory evoked responses. Prior to testing each horse, a process of judging its health and hearing was performed. Hearing was initially assessed behaviorally by observation of twitching of the ears and the Preyer reflex. Needle electrodes were then placed at three locations.

The active recording electrode was placed at the vertex, specifically, “a site on the face where the lines from the eyes to the base of the opposite ear cross” (p. 1445). Second, the reference electrode was placed at the bottom of the ear canal receiving the acoustic stimulus. Lastly, the ground electrode was placed on the pinna of the contralateral ear. Each pony or horse was restrained in a quiet stall with all equipment stable on a cart, which was then placed behind a wall. Acepromazine maleate was administered to keep each animal quiet during testing. One ear was tested, typically the right. Electrode impedance was between 2.5 and 3.5 kiloOhms for each animal. A bandpass filter of 0.3 to 8 kHz was used. Artifact reject systems as well as the 60-Hz notch filter were used. Typically 3,000 samples were recorded for each response but in some cases up to 8,000 samples were recorded in order to obtain a good result. Two recordings were taken to show reproducibility. A rarefaction click of 100 microsecond duration was used via headphones. Clicks were given at 55 per second in 10 dB steps from 10 dB HL to 90 dB HL (Marshall, 1985).

Of those tested, one horse had a threshold waveform at 20 dB HL. Two horses had one waveform visible below 50 dB HL, and four ponies had one below 40 dB HL. Fewer peaks were seen for the horses and ponies than in humans, dogs, and cats, but as the stimulus intensity increased, waves I through V were seen clearly. Amplitude of the waveforms increased as stimulus increased; among all horses and ponies the amplitudes were relatively low. Latency decreased as stimulus intensity increased; the average latencies were shorter for the ponies than for the horses. Interpeak latencies showed a slight decrease as stimulus intensity increased but no significant differences were found between the horses and ponies. Overall, the brainstem auditory evoked response test can

identify unilateral and bilateral deafness and differentiate between conductive and sensorineural hearing loss. A conductive loss shows normal latencies between waves but increased absolute latencies. Sensorineural hearing loss will show abnormal absolute latencies. Lastly, with complete deafness there will be no response (Marshall, 1985).

In another study, a three-year-old gelded sorrel and white overo American paint horse was examined due to a suspected hearing loss. He appeared unresponsive to noise and the owners had never heard him vocalize. Physically the gelding was in good condition and cranial nerve and other physical examinations showed everything to be within normal limits; however, the startle response was absent. A brainstem auditory evoked response was then performed (Harland, Stewart, Marshall, & Belknap, 2006).

The horse was sedated with detomidine hydrochloride. Three platinum electrodes were placed subdermally on the horse's head. The reference electrode was placed at the vertex, the active recording electrode was placed laterally at the most ventral portion of the ear canal that could be reached. The ground electrode was inserted at the same place as the active recording electrode but on the contralateral ear. A TDH-39 headphone was held against the pinna for the click stimulus to be presented. Clicks were delivered at 103 dB SPL at a rate of 20 clicks per second. The test was first conducted several times on a control horse to obtain a clear reading; it was then performed on the gelding. Two separate ipsilateral readings were taken and superimposed on each other to insure good reproducibility. As compared to the control horse, which had five clear peaks, the gelding did not display any peaks on the brainstem auditory evoked response confirming possible sensorineural deafness (Harland et al., 2006).

Mayhew and Washbourne (1990) analyzed brainstem auditory evoked responses in an unspecified number of horses. Horses were moderately sedated using detomidine hydrochloride. Electrodes were placed at the vertex and zygomatic processes of the temporal bones on the ipsilateral and contralateral side. Potentials were elicited with compression [*sic*] clicks at intensities ranging from 30 to 100 dB HHL [*sic*]. A white noise masker was presented in the ear contralateral to recording 10 dB below the compression click to prevent a response from the other ear and to block out external noise. High-pass filters were set at 200 Hz and low-pass filters were set at 2 kHz. For higher intensity clicks, 70 dB and above, 500 samples were averaged; for clicks of less intensity 1000 samples were averaged. For each dB level, at least two responses were recorded. Variations among horses were seen but similarities in latencies, amplitudes and morphology were observed. No latency delays were seen in waveforms, which suggests that sedation does not affect results. One mare had a lower amplitude wave V and a prolonged latency and a lesion was suspected on the brainstem. A thoroughbred filly that had signs of a progressive brain disorder showed asymmetry in the waveforms after wave III. It was also reported that as the stimulus intensity was decreased, the latency of the waves increased. These were the only findings discussed (Mayhew & Washborne, 1990).

Rolf and colleagues (1987) performed brainstem auditory evoked response testing on 10 healthy horses. Horses were given xylazine, glyceryl guaiacolate solution IV, thiamyal, halothane gas anesthesia and were restrained laterally. Alternating polarity 70 dB SPL click at a rate of 20 clicks per second were presented through an insert earphone using a hearing aid receiver with a frequency response of 100 to 6,000 Hz. Earphones were held in place with wax earplugs. Impedance measurements for the subdermal

electrodes were between 1 and 10 kiloOhms. The high-pass filter was set at 0.08 kHz and a low-pass filter was set at 3.2 kHz. At each intensity, a minimum of 500 clicks were presented; if the number of clicks was increased to 4,000 and no peaks were observed, the ear was labeled as yielding no response. Waveforms were recorded and analyzed starting at 136 dB SPL. Absolute latencies for each waveform were also measured.

Five peaks were observed across horses with an occasional late wave. Latencies were measured for waves I through V with the consensus that latencies decreased as stimulus intensity increased. No peaks were present for stimulus levels below 87 dB SPL. It was noted that this should not be considered auditory threshold due to the noisy environment testing was performed in. All latencies reported from the anesthetized horses were shorter in comparison to latencies received from horses tested at similar stimulus intensities that in other studies. It is not clear why this would occur; it is possible that wave I was incorrectly identified by the authors (see Table 1). According to the authors, waves I and V were well defined at intensities 117 dB SPL to 136 dB SPL while wave V was the most identifiable wave across intensities (Rolf et al., 1987).

Table 1

Absolute latencies

SPL (dB)	I	II	III	IV	V
136	1.36 ± 0.05 (2)	2.20 ± 0.2 (2)	3.06 ± 0.6 (2)	3.92 ± 0.05 (2)	4.71 ± 0.24 (2)
127	1.38 ± 0.3 (8)	2.40 ± 0.3 (7)	3.48 ± 0.4 (8)	4.32 ± 0.31 (8)	5.46 ± 0.72 (8)
117	1.60 ± 0.2 (4)	2.34 ± 0.02 (2)	3.42 ± 0.5 (4)	4.50 ± 0.63 (4)	5.48 ± 0.89 (4)
107	1.40 ± 0.2 (8)	2.50 ± 0.5 (8)	3.61 ± 0.27 (8)	4.47 ± 0.33 (8)	5.47 ± 0.65 (8)
97	1.74 ± 0.04 (2)	3.00 ± 0.02 (2)	3.65 ± 0.30 (2)	4.32 ± 0.17 (2)	5.06 ± 0.29 (2)
87	1.73 ± 0.6 (4)	2.60 ± 0.5 (6)	3.82 ± 0.40 (6)	4.80 ± 0.53 (5)	5.71 ± 0.39 (7)

Note. Recreated from "Auditory brain stem response testing in anesthetized horses," Rolf et al., 1987, *American Journal of Veterinary Research*, 48, p. 911. Copyright 1987 by American Veterinary Medical Association. Recreated with permission.
Latencies are recorded in ms.
Data are expressed as mean ± *SD* (number of horses tested at given intensity).
SPL – sound pressure level.

Studies After 2008

Relatively few recent reports have been published about brainstem auditory evoked responses in equines. This is surprising given the fact that instrumentation has improved and test protocols have evolved since the 1980s when the first reports were published. Specifically, use of insert earphones allows better accuracy in determining stimulus levels reaching the ear. Early studies used supra-aural earphones designed to fit over human pinnae. These phones had to be manually held in place near a horse's ear, eliminating any consistency in stimulus levels delivered to the ear.

Wilson et al. (2011) looked at brainstem auditory evoked responses in five young horses (5 to 8 years) and four old horses (17 to 22 years). Only the right ear was tested to reduce time needed under sedation. Before brainstem auditory evoked response testing

was performed, the Preyer reflex and review of health was completed showing that all horses did not have a hearing loss and were in good health. A 100 microsecond click stimulus was presented at levels ranging from 20 to 90 dB HL (approximately 50 to 120 dB peSPL). Both threshold (the lowest stimulus level with a repeatable wave V) and latencies were examined from the waveforms. Each horse had two recorded waveforms at each stimulus level, which were averaged together; these were then compared to previously published studies on hearing in adult horses. Results showed that the younger horses had results within normal limits and the older horses had results outside of normal limits, indicating a hearing loss. When looking at latencies and amplitudes between waves I and V at the 90 dB HL stimulus, two of the horses were outside of normal limits, indicative of partial hearing loss suspected to be due to presbycusis. Normal limits were not specifically stated; when looking at the graphs provided, it appeared that wave I for the youngest horse (6 years old) occurred between 1 and 2 msec, while wave I for the oldest horse (22 years old) occurred shortly after 2 msec. Wave V for the young horses occurred shortly after 4 msec, while wave V for the older horse occurred shortly after 5 msec (Wilson et al., 2011).

In a retrospective a study by Aleman et al. (2014), brainstem auditory evoked response results were related to specific diseases or pathologies. A total of 76 horses of multiple breeds were tested, ages ranging from 1 to 28 years. Testing of the horses occurred over a period of 31 years; all were tested because of suspected hearing loss associated with a disease process. The most common observations associated with disease or pathology were altered behaviors including startle to environment, head tilt,

leaning to one side, gait issues, corneal ulceration, and dysphagia. Headshaking, seizures, inability to stand, and collapsing were among other behaviors recorded.

Horses were placed in stocks for recording and given xylazine hydrochloride. Insert earphones were placed into the canal for most of the brainstem evoked response recordings, while headphones were used for early recordings. Subdermal needle electrodes were placed at the vertex, left mastoid, right mastoid and on the dorsal midline at the level of C2 vertebra. Each recording consisted of at least 400 samples. An alternating broadband click stimulus at 90 dB nHL was presented to each ear individually while masking noise was presented to the contralateral ear. All waveforms were recorded twice. No other parameters were reported regarding stimulus level, equipment used, click rate, etc. Criteria used to distinguish abnormal brainstem auditory evoked response results from normal included absence of brainstem auditory evoked response peaks, absence of peaks following wave I or II, and abnormal interpeak latencies. These abnormalities were used to localize lesions of the cranial nerve, cranial brainstem, and caudal brainstem depending on where the abnormality occurred (Aleman et al., 2014).

The authors concluded that 57 horses had hearing loss; among these, 42 were bilateral and 15 were unilateral. Of those horses with a bilateral hearing loss, 11 were diagnosed with multifocal brain disease, 10 with temporohyoid osteoarthropathy, one with strictly vestibular issues, and one without an etiology. Among those with a unilateral hearing loss, the common causes included temporohyoid osteoarthropathy, otitis media, and multifocal brainstem disease. Responses from horses with otitis media consisted of an entirely absent brainstem auditory evoked response. The authors concluded that it cannot be determined whether a horse has a hearing loss solely based on response to loud

sounds; a hearing loss may still be present. Signs of hearing loss varied among horses dependent on the disease and whether it was related to bilateral or unilateral hearing loss. This shows the importance of completing a brainstem auditory evoked response diagnostic as part of an evaluation for a horse exhibiting behavioral alterations (Aleman et al., 2014).

Aleman et al. (2008) reviewed whether auditory abnormalities exist in horses with temporohyoid osteoarthropathy. Brainstem auditory evoked responses were recorded in 11 horses with temporohyoid osteoarthropathy and compared to 8 adult control horses. Horses with temporohyoid osteoarthropathy were diagnosed in one of three ways: endoscopy, computed tomographic (CT) evaluation, or radiography. Brainstem auditory evoked responses were recorded over a two-year period. Parameters used included 400 samples within a 10 msec window. Two waveforms were recorded at each presentation level. An alternating polarity broadband click stimulus was used at 90 dB nHL at a rate of 10.1 Hz. White noise masking was used in the opposite ear at 60 dB nHL in order to avoid stimulation of the other ear. For each response, peaks were labeled and interpeak values for I-III, III-V, and I-V were determined. Criteria used to determine abnormalities were as follows: absence of brainstem auditory evoked response peaks indicated complete hearing loss and increased peak latencies or difficult peak identification indicated partial hearing loss.

Of the 11 horses with temporohyoid osteoarthropathy, 5 had absent brainstem auditory evoked responses unilaterally (on the affected side) with partial hearing loss (increased peak latencies or difficult peak identification) contralaterally. Of the remaining, 4 had unilateral brainstem auditory evoked response absences with a present

recording from the other ear, and 2 had partial hearing loss unilaterally with a present recording from the other ear. Figures 2 and 3, and Table 2 represent results obtained from the study including a normal brainstem auditory evoked response (see Figure 2), an absent brainstem auditory evoked response (see Figure 3), and peak latencies as well as interpeak intervals (see Table 2) (Aleman et al., 2008).

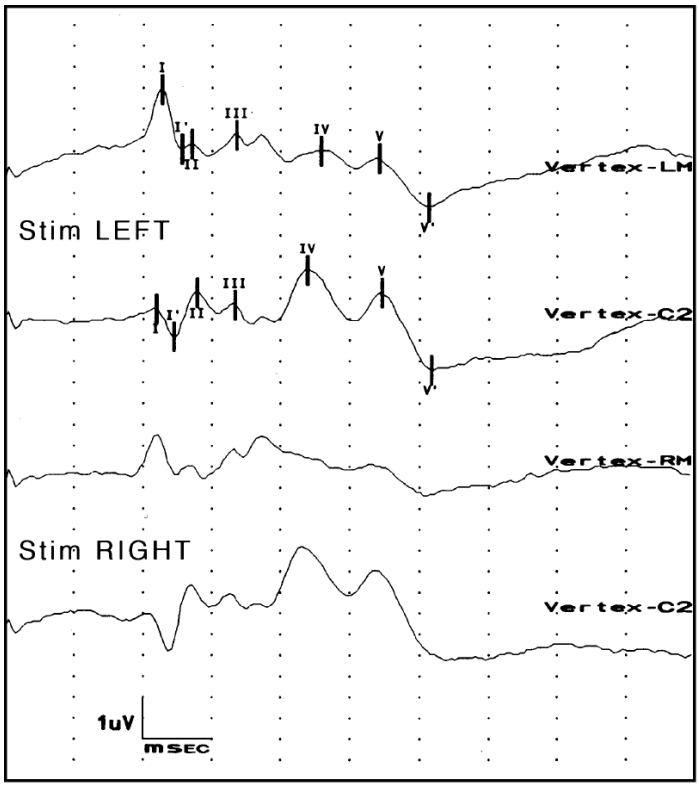

Figure 2. Normal brainstem auditory evoked response. Reprinted from "Brainstem auditory-evoked responses in horses with temporohyoid osteoarthropathy," Aleman et al., 2008, *Journal of Veterinary Internal Medicine*, 22, p. 1197. Copyright 2008 by the American College of Veterinary Internal Medicine. Reprinted with permission.

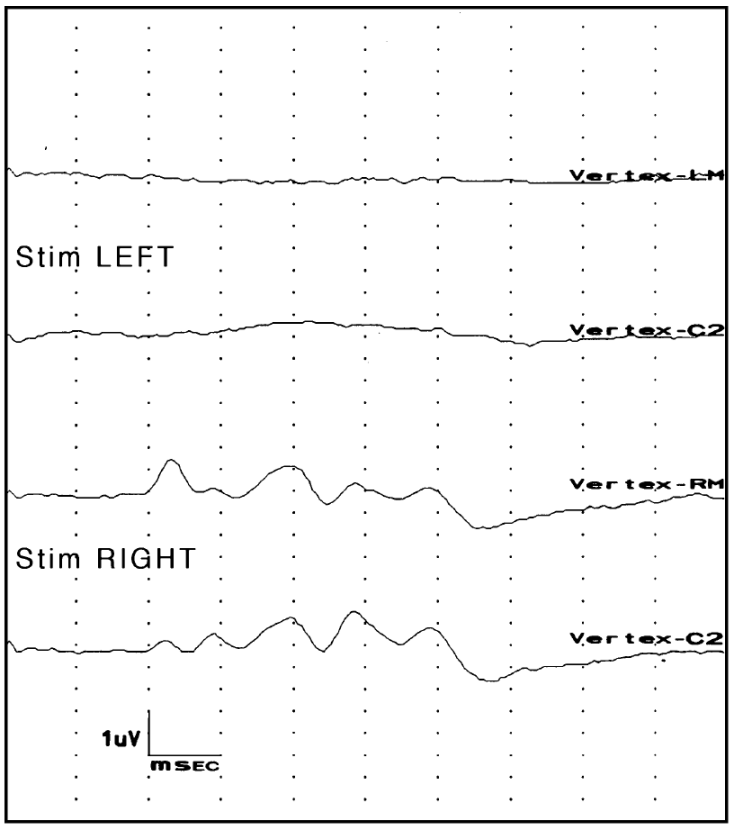

Figure 3. Absent brainstem auditory evoked response. Reprinted from "Brainstem auditory-evoked responses in horses with temporohyoid osteoarthropathy," Aleman et al., 2008, *Journal of Veterinary Internal Medicine*, 22, p. 1198. Copyright 2008 by the American College of Veterinary Internal Medicine. Reprinted with permission.

Table 2

Peak latencies and interpeak latencies

	I	III	IV	V	I-III	III-V	I-V
Left ear							
V-M							
Control (8)	2.2 (2.14-2.28)	3.63 (3.33-3.76)	4.56 (4.34-4.83)	5.35 (5.08-5.52)	1.41 (1.08-1.56)	1.69 (1.64-2.06)	3.14 (2.94-3.32)
THO(7)	2.24 (2.17-3.57)	3.5 (3.43-4.79)	4.52 (4.25-5.04)	5.54 (5.25-6.77)	1.22 (1.12-1.44)	2.03 (1.66-2.33)	3.22 (3.02-3.64)
V-C2							
Control (8)	2.1 (2.03-2.19)	3.64 (3.31-3.76)	4.32 (4.23-4.66)	5.38 (5.21-5.52)	1.51 (1.14-1.57)	1.73 (1.61-1.74)	3.23 (3.14-3.32)
THO(7)	2.18 (2.11-3.73)	3.59 (3.32-4.82)	4.5 (4.25-4.66)	5.44 (5.1-6.7)	1.28 (1.09-1.48)	1.94 (1.62-2.22)	3.22 (3.14-3.32)
Right ear							
V-M							
Control (8)	2.2 (2.18-2.34)	3.66 (3.64-3.8)	4.51 (4.26-4.95)	5.39 (5.22-5.61)	1.46 (1.1-1.52)	1.72 (1.58-2.08)	3.19 (3.04-3.28)
THO (7)	2.27 (2.15-2.33)	3.54 (3.37-3.99)	4.68 (4.54-4.97)	5.53 (5.35-5.76)	1.24 (1.22-1.77)	1.95 (1.77-2.14)	3.36 (3.12-3.57)
V-C2							
Control (8)	2.08 (2.07-2.24)	3.68 (3.25-3.85)	4.4 (4.29-4.95)	5.41 (5.24-5.59)	1.49 (1.18-1.68)	1.71 (1.56-2.12)	3.21 (3.04-3.42)
THO (7)	2.25 (2.19-2.27)	3.57 (3.41-3.98)	4.56 (4.47-5.08)	5.63 (5.35-5.85)	1.33 (1.24-1.74)	1.94 (1.68-2.08)	3.4 (3.2-3.42)

Note. Recreated from "Brainstem auditory-evoked responses in horses with temporohyoid osteoarthropathy," Aleman et al., 2008, *Journal of Veterinary Internal Medicine*, 22, p. 1200. Copyright 2008 by the American College of Veterinary Internal Medicine. Recreated with permission.

THO, temporohyoid osteoarthropathy

Latencies are recorded in (ms)

V-M, vertex to ipsilateral mastoid, V-C2, vertex to C2

Magdesian et al. (2009) evaluated deafness in American paint horses by examining multiple areas including phenotype, clinical findings, endothelin B receptor, and brainstem auditory evoked responses. Three groups of horses were included within in the study: those that were confirmed deaf, those that were suspected of being deaf, and those that did not have a hearing loss to serve as a control group. Sedatives were administered to each horse tested. Subdermal needle electrodes were placed according to "a hospital protocol for brainstem auditory evoked response in horses" (p. 1205). Multiple recordings were performed at each intensity, but no parameters were included for recording of the waveforms aside from electrode placement. Brainstem auditory evoked response results indicated absence of waveforms in those that were confirmed deaf and in 3 foals within the suspected-deaf group; all 3 foals also had confirmed overo lethal white foal syndrome. Each of the 3 foals had blue irises and more than 98% of their coat was white.

Gama et al. (2016) performed brainstem auditory evoked potentials to evaluate the auditory pathway in 21 clinically healthy horses. The purpose of the study was to standardize brainstem auditory evoked potential testing in horses in Brazil. Each animal fasted for 12 hours prior to the procedure. They were then sedated intravenously with detomidine hydrochloride and testing was initiated 10 minutes post injection. Needle electrodes were placed subcutaneously at four points: the base of each ear, the forehead, and the vertex. A band-pass filter of 200 to 3000 Hz was used. Rarefaction clicks with a duration of 2 ms [*sic*] and an intensity of 90 dB were presented in the test ear with a masking noise at 40 dB in the contralateral ear. For each ear at least two sets of 500

stimuli were recorded. Absolute latencies of waves I, III, and V were measured as well as interpeak latencies of I-III, III-V, and I-V.

No significant differences were found between males and females and only one significant difference, wave III latency, was found between right and left sides. A table of mean, minimum, maximum values and standard deviations of the latencies of the waves at 90 dB from the study is included below (see Table 3). Results were obtained for standardization of latencies for future diagnosis of hearing disorders in horses (Gama et al., 2016).

Table 3

Latency table

Latency	*M*	Minimum-Maximum (ms)	*SD*
I	2.24	2.10-2.34	0.06
II	2.74	2.58-2.92	0.08
III	3.61	3.36-3.88	0.12
IV	4.61	4.22-4.86	0.17
V	5.49	5.24-5.92	0.15
I-III	1.37	1.16-1.64	0.11
III-V	1.88	1.46-2.18	0.16
I-V	3.26	2.98-3.70	0.16

Note. Recreated from "Brainstem auditory evoked potentials in horses", Gama et al., 2016, *Clinic and Surgery*, 46, p. 684. Copyright 2016 by Clinic and Surgery. Recreated with permission.

Summary

It is important to ascertain the hearing status of horses who may be at risk for hearing loss. Kane (2015) stated that horses that are deaf do not become unable to train, but a different technique must be used. Many riders and trainers aren't aware of their horses' deafness, which can lead to challenges, but with trainer and rider knowledge, visual and tactile cues can be used for deaf horses. Along with these techniques, new and

unfamiliar environments should be avoided as much as possible; these horses should be approached in their visual field, and it should be noted that they startle more easily.

While several researchers have explored brainstem auditory evoked response results in the equine population, only one study performed by Wilson and colleagues (2011) explored brainstem auditory evoked response results relating to presbycusis. Much of the equine literature is dated prior to 2008 and is limited in terms of the explanation of parameters used to record the brainstem auditory evoked response waveform, and some failed to report parameters entirely (Aleman et al., 2014; Magdesian et al., 2009). Masking was sometimes used inappropriately in recordings (Aleman et al., 2008; Aleman et al., 2014; Mayhew & Washbourne, 1990). One study used a large bandpass filter setting, up to 8 kHz, and a fast click rate of 55 clicks per second (Marshall, 1985). Another study used supra-aural headphones rather than insert earphones for some recordings (Aleman et al., 2014). All studies had inconsistent parameters or parameters were not reported at all, and some used dB HL or dB nHL to describe stimulus levels; these decibel scales were developed to describe human hearing levels and cannot be generalized to the equine population (Aleman et al., 2008; Aleman et al., 2014; Marshall, 1985; Wilson et al., 2011). One study even reported decibel levels in dB HHL, a scale not described and not reported elsewhere (Mayhew & Washbourne, 1990). Another study excluded the dB reference value used (Gama et al., 2016). All of the above makes replication and comparison of test results difficult. In order to analyze the equine brainstem auditory evoked response correctly, standardization of protocol should be established. The present study attempted to use current instrumentation and

testing protocols to obtain brainstem auditory evoked responses in young, normal equines and compare the results to those of healthy older equines.

CHAPTER III

METHODOLOGY

Participants

Institutional Animal Care and Use Committee (IACUC) approval for research was obtained prior to recruiting and testing. The participant group contained 10 horses. The horses were grouped by age into a young group (4 to 7 years, n=4) and an old age group (20 to 31 years, n=6). The participants had no history of noise exposure and no suspected or diagnosed disease that affects the auditory system, as reported by the horse owners. Each horse owner signed a consent form that described the test procedure. All testing was done on the owner's premises. Horses were tested in either crossties or in an enclosed area. All horse owners received a pair of Cashel foam earplugs to practice inserting and removing from the horse's ear prior to testing to desensitize the horse to having an object in its ear.

Preparation of Equines

On the day of testing each equine received a visual otological examination prior to beginning testing to assess ear canal clarity and health. A thin film of lidocaine topical cream (lidocaine 2.5%/prilocaine 2.5%) was used as an analgesic at the site of each electrode placement. Rhythmlink disposable subdermal needles with a 13 mm length and 0.4 mm diameter were placed at three different locations on the horse: below the tragus on the right ear, middle of the forehead (Fz), and on the neck (see Figure 4). Once the

lidocaine/prilocaine cream was rubbed in and absorbed, the insertion of the electrodes was performed while standing at the right shoulder of the horse. Electrodes were inserted gently just beneath the skin by pinching a small portion of skin and inserting the electrode with the opposite hand. The neck was used as a ground/common electrode site, the test ear was the negative (inverting) electrode site, and the forehead was the positive (non-inverting) electrode site. The owner was asked to insert the Cashel earplug into the ear being tested. A wrap was then placed around the horse's pinna to secure earphone placement during testing and another wrap was placed around the neck to hold electrode cables in place (see Figure 4).

Figure 4. Placement of electrodes, insert earphone, and wrap on test subject

Recording Procedure

The Intelligent Hearing Systems (IHS) USB box with Smart EP software version 5.10 was used connected to an HP laptop computer with a Windows 7 operating system. Electrode impedance was checked with the 2-channel Opti-Amp Power Transmitter prior to each recording and impedance was kept between 1 and 3 kiloOhms at electrode sites.

A 100 microsecond broadband click with 12,000 Hz bandwidth power spectrum was used to elicit a response. A click stimulus produced by the computer was directed into the ear via a Cashel foam earplug, which was modified to hold an ER1-14A insert earphone, or ER-2, as the software designates it. Stimuli were presented at a rate of 21.1 clicks per second using a rarefaction polarity. Stimulus intensities included 100 dB peSPL, 90 dB peSPL and down in 10 dB increments until wave V was no longer visible. If an unclear waveform was recorded at 100 dB peSPL, the stimulus intensity was increased to 110 dB peSPL. Only right ear recordings were taken in order to reduce the duration of test time. The amplifier had an absolute gain setting of 100,000 to 150,000 and was set to record in mircovolts (μV). A high-pass filter was set at 100 to 150 Hz and a low-pass filter was set at 3 kHz. Artifact reject was set to 35% and the line filter setting was off. For each recording at least 500 sweeps were collected. At each stimulus level, at least two recordings were collected in a 12 ms window to ensure reproducibility.

Analysis

Latencies of waves I, III, and V, interpeak intervals of I-III, III-V, and I-V, and the peak-to-trough amplitude of wave V were identified at the highest intensity level. The lowest level at which wave V was present and repeatable for each equine was defined as estimated hearing threshold; wave V peaks were identified and agreed upon by three individuals experienced in identifying BAER waves. When two or three repeated waves were similar they were averaged to measure latencies and amplitudes. If repeatability was poor, the wave with the best overall morphology was used to determine latencies and amplitudes. Means and standard deviations were calculated for each group.

CHAPTER IV

RESULTS

Data were collected from 10 equines ages 5 to 31, separated into two groups, 6 old equines (ages 20 to 31) and 4 young equines (ages 5 to 7). Data obtained from one of the older horses was not included in the data analysis due to excessive noise from tooth grinding that obscured the waveforms. A summary of breed, age, and sex of each equine included in the data analysis can be found in Table 4. Brainstem auditory evoked responses were obtained and used for analysis. Waveform morphology for most subjects from both groups was generally poor with the number of artifacts occurring ranging from 0 to 252 during each run of at least 500 clicks.

Table 4

Age, breed, and sex of each test subject

Participant #	Age	Breed	Sex
1	5	Morgan	Female
2	6	Morgan	Male
3	7	Andalusian Cross	Female
4	7	Dutch Warmblood/Thoroughbred	Male
5	20	Paint	Male
6	24	American Quarter Horse	Female
7	25	American Quarter Horse	Female
8	31	Morgan	Male
9	31	Appaloosa/American Quarter Horse	Female

Absolute latencies for waves I, III, V and interpeak latencies for waves I-III, III-V, I-V were obtained from the 100 dB SPL waveforms and descriptively analyzed (see Table 5). Peak-to-trough amplitudes for waves I, III, and V were calculated and are

reported in Table 6. Thresholds were estimated from the lowest intensity level at which a wave V was judged to be present (in 10 dB step sizes). Mean thresholds and the ranges of thresholds for each test group can be found in Table 7. Figure 5 illustrates threshold relative to age, Figure 6 illustrates wave V latency relative to age, and Figure 7 illustrates wave V amplitude relative to age. Raw data for all subjects can be found in Appendix C.

Due to the small sample size and concerns regarding violating assumptions required for the parametric analyses, a nonparametric statistical hypothesis test, Mann-Whitney U, was performed. The Mann-Whitney U test indicated no statistically significant differences between the two groups; statistical values for the Mann-Whitney test are reported in Table 8.

Table 5

Mean absolute latencies, mean interpeak latencies, and standard deviations for both test groups

Subject group	I	III	V	I-III	III-V	I-V
Young Horse	2.35 (0.04)	3.57 (0.04)	5.60 (0.15)	1.22 (0.03)	2.04 (0.14)	3.25 (0.12)
Old Horse	2.31 (0.10)	3.55 (0.20)	5.40 (0.29)	1.24 (0.28)	1.86 (0.30)	3.09 (0.27)

Note. SD is reported in parentheses

Table 6

Mean peak-to-trough amplitudes at 100 dB SPL for both test groups

	I	III	V
Young Horse	0.31 uV	0.15 uV	0.34 uV
Old Horse	0.26 uV	0.37 uV	0.21 uV

Table 7

Range of thresholds, mean thresholds, and standard deviations for both test groups

	Range of thresholds	Mean threshold	*SD*
Young Horse	60-80 dB SPL	70 dB SPL	8.2
Old Horse	60-90 dB SPL	74 dB SPL	13.4

Table 8

Z-values and significance from statistical analysis

	Mann-Whitney U (*z*-value)	Mann-Whitney significance (one-tailed)
Wave I Latency	-.496	.730
Wave III Latency	-.747	.556
Wave V Latency	-.980	.413
Wave I-III Latency	-.490	.730
Wave III-V Latency	-.980	.413
Wave I-V Latency	-.738	.556
Wave I Amplitude	-.615	.556
Wave III Amplitude	-.1.845	.063
Wave V Amplitude	-.980	.413
Wave V Threshold	-.509	.730

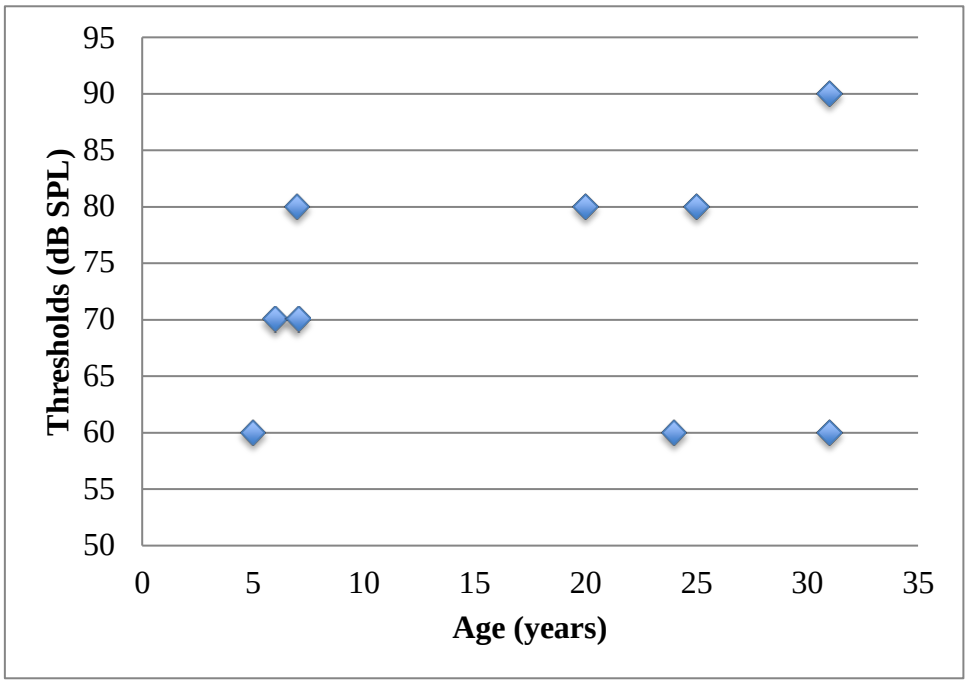

Figure 5. Wave V thresholds relative to age

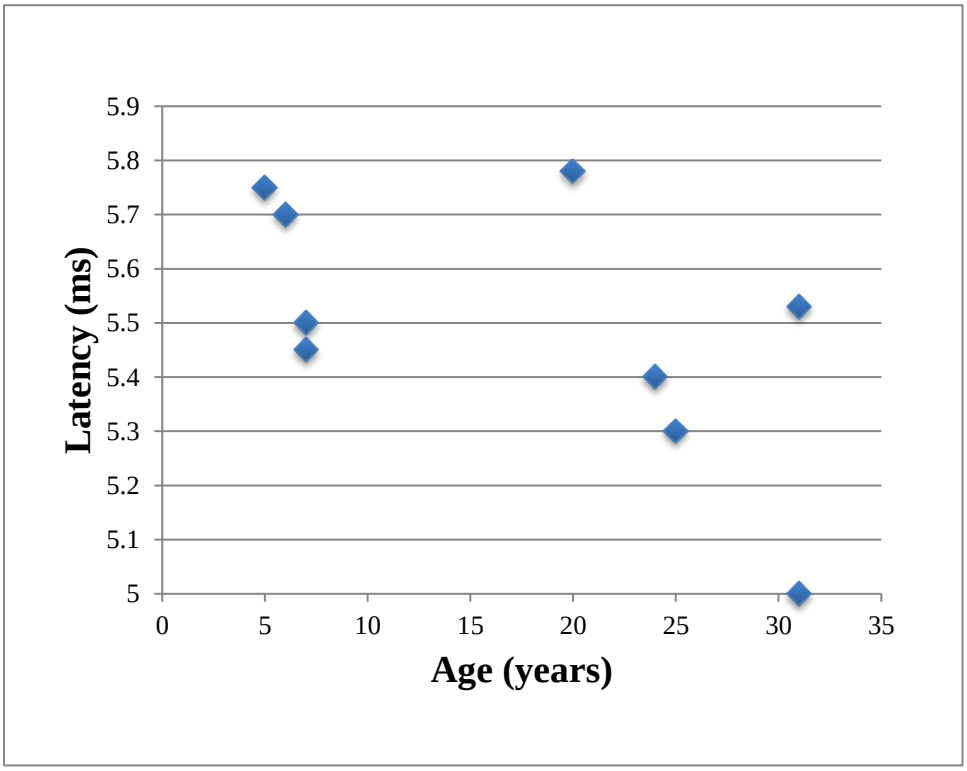

Figure 6. Wave V latencies relative to age

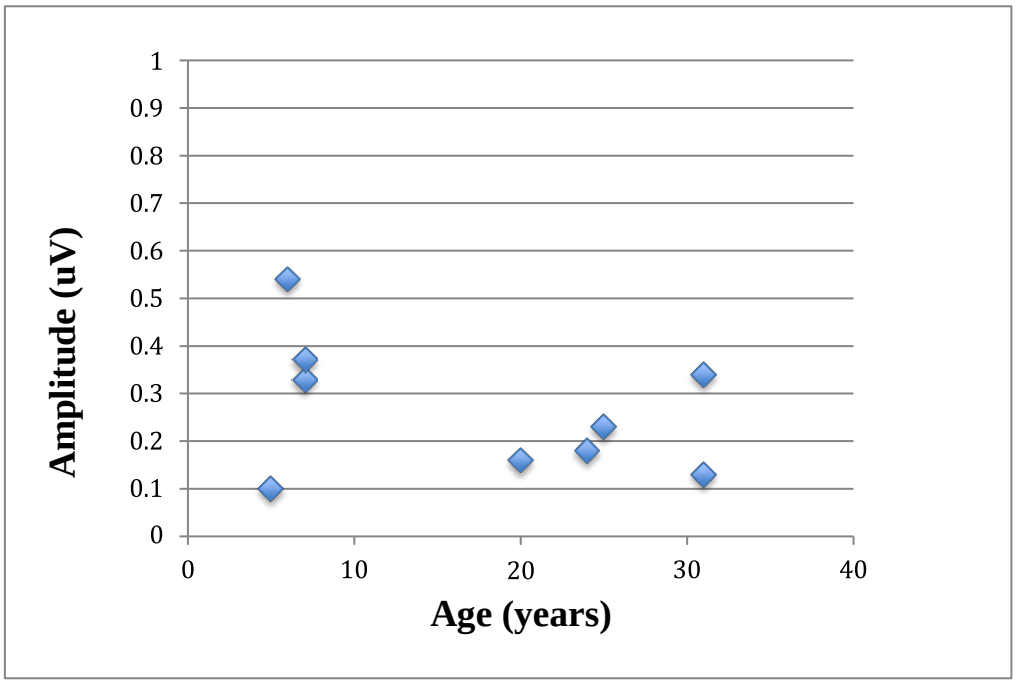

Figure 7. Wave V amplitudes (peak-to-trough) relative to age

CHAPTER V

DISCUSSION

The purpose of this study was to examine the effects of age on brainstem auditory evoked responses (BAERs) in equines that were not exposed to noise, and in result would not have a noise-induced hearing loss. Specifically, threshold, peak latencies, peak amplitudes, and morphology were analyzed for two groups: young and old equines. It was hypothesized that the older group of horses would exhibit poorer thresholds, poorer morphology, increased latencies, and decreased amplitudes of waves compared to the younger group.

Summary of Results

The results of this study show that brainstem auditory evoked responses can be identified and replicated in older horses and no differences were observed between young and old horses (see Figure 8). There were no statistically significant differences in mean threshold between the two groups. No difference was found for absolute latencies and interpeak latencies between the two groups. Waveform morphology was poor for both the old and young population compared to those seen in humans, primarily because of high numbers of artifact during recording; there was little difference between the two populations. Amplitude differences were also not notable between the two populations.

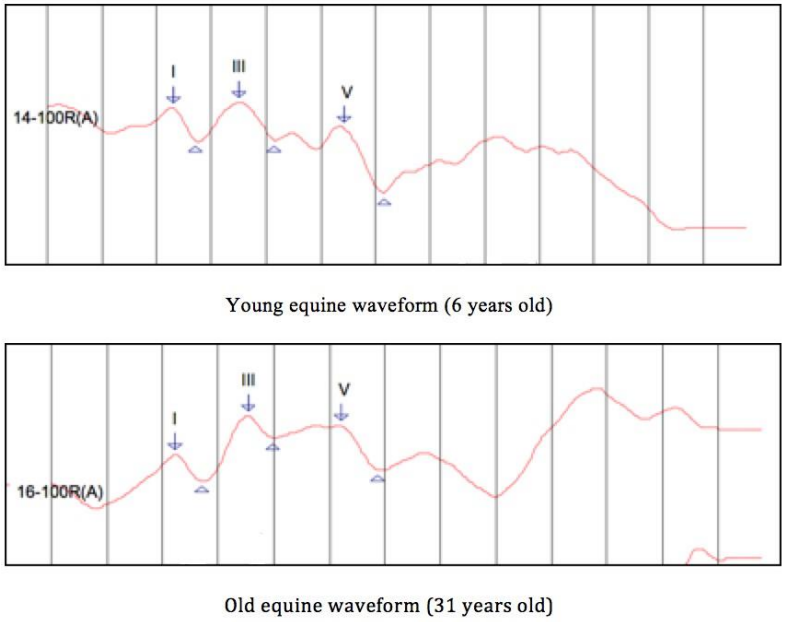

Figure 8. Examples of waveforms from young and old equines

Results Relative to the Literature

Threshold results of this study are in agreement with previously reported results. Rolf and colleagues (1987) indicated a threshold consistent with the highest reported threshold from the current study; they reported an 87 dB SPL threshold as the lowest recorded threshold in a group of horses of varying age, breed and sex. The current study had a threshold of 90 dB SPL in one of the old test subjects. Mayhew and Washbourne (1990) reported thresholds as low as 30 dB HHL [*sic*] in a group of horses of unknown age; one threshold from the current study was obtained at 60 dB SPL. Wilson and colleagues (2011) performed brainstem auditory evoked response testing on nine geldings ages 5, 6, 6, 7, 8, 17, 18, 21 and 22 years and reported a threshold of 25 dBnHL in one of the younger horses and 50 dBnHL in one of the older horses. In the current study a threshold of 60 dB SPL was collected for a young horse and 90 dB SPL was collected for

an old horse (for human results, dBnHL is approximately 30 dB better than dB SPL so it would appear that the threshold results across studies are consistent). Marshall (1985) reported a “recognizable” brainstem auditory evoked response at 20 dB HL in one horse of unknown age, which is presumably within 10 dB of the low threshold of 60 dB SPL in the current study.

Regarding absolute and interpeak latencies, there is limited variability of agreement among studies. Shorter latencies have been reported compared to those from the current study (Harland et al., 2006; Marshall, 1985; Rolf et al., 1987), but only for wave I. Latencies similar to those of the current study have been reported across waves I, III, and V in multiple studies (Aleman et al., 2008; Aleman et al., 2014; Gama et al., 2016).

Most research thus far has used anesthetization or some form of sedation during testing (Aleman et al., 2008; Aleman et al., 2014; Gama et al., 2016; Harland et al., 2006; Magdesian et al., 2009; Marshall, 1985; Mayhew & Washbourne, 1990; Rolf et al., 1987; Wilson et al., 2011). A summary of previous studies and the key findings can be viewed in Table 9.

Table 9

Key methodology with related findings from previous and current research

Study	Participants	Sedation Used?	Age (years)	Stimulus Level	Latency Findings	Threshold Findings
Aleman et al., 2014	Control group – 8 horses quarter horse, thoroughbred, and warmblood breeds	YES	5-20	90 dB	• Wave I 2.21, wave III 3.61, wave V 5.36 • Wave I-III 1.40, wave III-V 1.74, wave I-V 3.14	NR
Aleman et al., 2008	Control group – 8 horses quarter horse, thoroughbred, and warmblood breeds	YES	5-20	90 dB	• Wave I 2.2, wave III 3.63, wave V 5.35 • Wave I-III 1.41, wave III-V 1.69, wave I-V 3.14	NR
Gama et al., 2016	21 horses – 13 males and 8 females	YES	2-12	90 dB	• Wave I 2.4, wave III 3.61, wave V 5.49 • Wave I-III 1.37, wave III-V 1.88, wave I-V 3.26	NR
Harland et al., 2006	1 control horse	YES	NR	75 dB SPL	• (Approximated from figure) Wave I 1.8, wave III 3.5, wave V 4.5	NR
Magdesian et al., 2009	One control group consisting of paint and pinto	YES	NR		NR	NR
Marshall, 1985	10 horses	YES	NR	90 dB	• Wave I 1.44, wave III 3.81, wave V 5.87	• "Recognizable" BAER at 30 dB HL in one horse, 2 horses measureable response <50 dB HL
Mayhew & Washbourne, 1990	NR	YES	NR	NR	NR	• Thresholds as low as 30 dB HHL [*sic*]
Melvin (current study)	10 horses – morgan, andalusian cross, dutch warmblood, thoroughbred, appaloosa, paint, quarter horse, both sexes	NO – 9 horses YES – 1 horse	5-31	100 dB SPL	• Wave I 2.31, wave III 3.84, wave V 5.99 (old group) • Wave I-III 1.29, wave III-V 2.14, wave I-V 3.28 (old group) • Wave I 2.36, wave III 3.56, wave V 5.72 (young group) • Wave I-III 1.2, wave III-V 2.16, wave I-V 3.36 (young group)	• Old group mean threshold: 74 dB SPL • Young group mean threshold: 70 dB SPL
Rolf et al., 1987	10 horses – quarter horse, standardbred, grade horse, thoroughbred, appaloosa, saddlebred, both sexes	YES	2-9	107 dB SPL	• Wave I 1.4, wave III 3.61, wave V 5.47	• 87 dB SPL lowest reported threshold
Wilson et al., 2011	9 geldings – 7 standardbred, 2 thoroughbred	YES	5-22	NR	• Specific latencies and absolute latencies NR	• Old group threshold: 50 dBnHL • Young group threshold: 25 dBnHL

Note. NR indicates not reported

At first glance, it appears that threshold levels obtained in the current research do not agree with those reported in the literature but this is due to different reference values being used for stimulus levels. Sound pressure level is the amount of sound energy relative to a reference pressure. The calculation for dB sound pressure level (SPL) is equal to 20 times the log of the ratio of an observed sound pressure level to the reference sound pressure level of 20 microPascals (Stach, 2003). This decibel value represents the softest pressure that is audible in the frequency region where hearing is most sensitive (Schlauch & Nelson, 2015). The level of audibility in dB SPL varies across frequencies.

The dB Hearing Level (HL) scale was created to normalize the curve of audibility to represent hearing loss on an audiogram. The dB HL scale is used for testing human hearing levels for pure tones (single frequencies); 0 dB HL is the softest audible sound heard 50 percent of the time by a group of normal hearing humans. This is a normalized value used to compare thresholds in humans (Kramer, 2008). To convert dB HL to dB SPL the ANSI reference value for the specific frequency is added to the dB HL value. For example, the dB SPL value for insert earphones at 2,000 Hz is 11.5, so if a threshold were 0 dB HL at 2,000 Hz, the threshold would be 11.5 dB SPL (Kramer, 2008).

A similar value, dBnHL, is a behaviorally determined normalized value (Sharma, Purdy, & Bonnici, 2003) for a transient stimulus such as a click. The dB SPL value is a higher number than the dB HL value for any given threshold, which explains why when the two scales are compared between studies, the numbers between studies seem incongruent. Sharma et al. (2003) reported on the correction factor for converting dB nHL to dB SPL; results from their research and previous studies indicate a 25 to 30 dB correction factor. For example, the threshold of 25 dB nHL reported by Wilson and

colleagues (2011) in their young test group with the conversion of 30 dB is 55 dB SPL, which is in line with the 60 dB SPL threshold collected in the current study from one of the young test subjects. Since dB HL and dB nHL have been normalized based on hearing in humans, it is not appropriate to use either of those scales for brainstem auditory evoked response testing in animals.

Strengths and Limitations of the Study

Multiple difficulties were encountered in the present study. While testing, many of the horses produced a significant amount of artifact. This is likely due to the ten muscles around the external ear and their ability to rotate the external ear 180 degrees to localize sound (Griffin, 2017). For one subject, excessive artifact was due to grinding of the teeth and the data from that individual were not included in analysis. Another horse was unable to be tested due to lack of cooperation. This horse was rescheduled for testing at a later date, at which time the horse was sedated with .6 cc of Dormosedan by an attending veterinarian. Differences in sedated versus non-sedated waveforms from two different horses can be seen in Figure 9. Multiple artifacts were present during each test run for all subjects but were significantly less for the one horse that was sedated. High artifact levels made replication of waveforms difficult. Finding test subjects also proved to be quite difficult. Many horse owners were not enthusiastic about the testing process, specifically the use of subdermal needle electrodes.

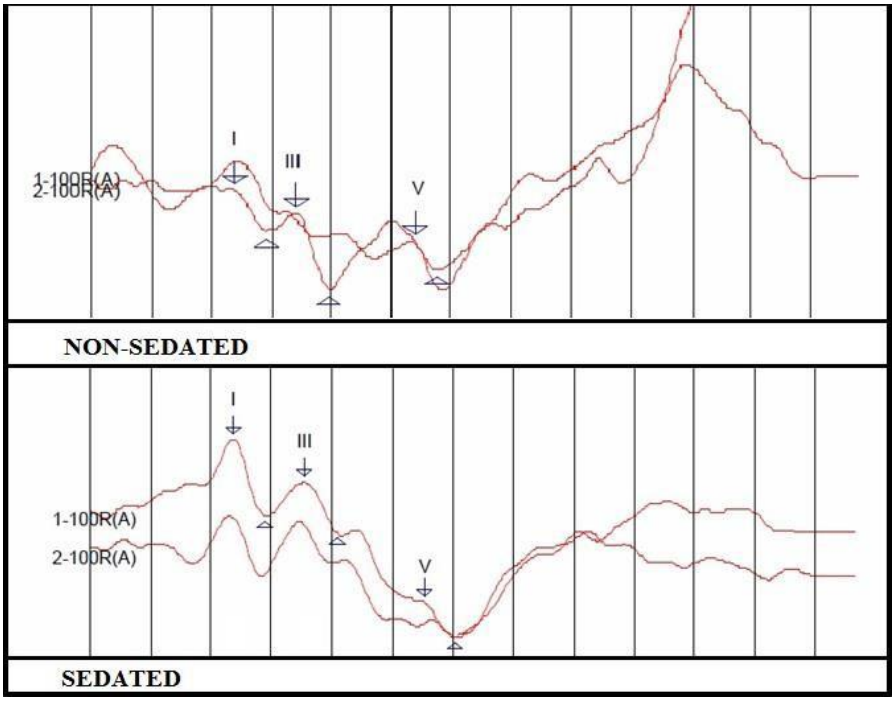

Figure 9. Examples of waveforms from a sedated horse and a non-sedated horse

Implications and Future Directions

Two test groups of equines, old and young, were chosen to examine the effects of age on the brainstem auditory evoked response. Equines without any previous noise exposure and no suspected or diagnosed ear disease were chosen in order to control for hearing loss. In humans, it is very difficult to test a population of older individuals solely to determine the effects of age on hearing because other factors including noise exposures and drug effects cannot be ruled out as impacting the hearing loss. With the insignificant differences viewed between the two test groups in this study, it may be possible that what is considered age-related hearing loss, or presbycusis, in humans may be the cumulative effect of noise exposure, ototoxicity effects, and other environmental effects occurring over a lifespan. It is possible that purely age-related effects cannot be studied in humans, and that in animals not exposed to the same environmental conditions, age-related changes in hearing may not be as pronounced. For example, studies of dogs indicate that

hearing loss and morphological changes related to aging do occur in a manner similar to that for humans (Shimada et al., 1998; ter Haar at al., 2008). It may be that dogs are more likely than horses to be exposed to the same types of environmental conditions such as noise and environmental toxins as their human owners, resulting in a similar pattern of age-related hearing loss. Further study of hearing in aging animals may provide clues to the etiology of presbycusis in the absence of other factors.

It is also possible that the click used to elicit the brainstem response in this study did not capture the frequency range in which presbycusis is most prevalent in equines. Due to the shape of the cochlea, presbycusis affects the basal region of the cochlea first (Schuknecht, 1974). The rapid onset click used within this study produces a waveform that reflects activity primarily from 1,000 Hz to 4,000 Hz (Hall, 2007). Heffner and Heffner (1983) reported that horses' hearing range is from 55 Hz to 33,500 Hz with the most sensitive range from 1,000 Hz through 16,000 Hz and a dip (worsening) at 4,000 Hz. In contrast, human hearing ranges from 20 to 20,000 Hz with the most sensitive range from 2,000 Hz to 5,000 Hz and good sensitivity persisting through the 100 Hz to 10,000 Hz range (Gelfand, 1998).

Future research should explore test-retest reliability of brainstem auditory evoked response testing in equines. Use of other auditory stimuli, such as tonebursts, should be explored to capture more frequency-specific information and to determine whether results would differ due to the contrast in horse and human hearing ranges. Also, in order to limit the amount of artifact, get more accurate and replicable waveforms, and reduce test duration, it is recommended that further research explore the feasibility of anesthetization/sedation of all test subjects. In addition, a follow-up study could compare

a sample of horses exposed to noise to the results of the current study to examine the effects of noise on the brainstem auditory evoked response (for example, for horses used for mounted shooting competitions).

Conclusions

In summary, it is possible to obtain brainstem auditory evoked responses results on older horses. Results indicate no differences in threshold, latency, or amplitude between young and old horses. Testing is possible on horses that are not sedated or anesthetized; however, more artifact is encountered during testing and the morphology of the waveforms are typically not as clear as desired. Insignificant differences between the two test groups indicate that it may be that what is considered presbycusis in humans is actually the result of complex interaction of other factors such as noise, ototoxicity, and other environmental effects. It is also possible that the click used in the current study did not capture the frequency range most affected by aging within horses.

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APPENDIX A

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) APPROVAL

UNIVERSITY of NORTHERN COLORADO

IACUC Memorandum

To:	Dr. Katie Bright
From:	Laura Martin, Director of Compliance and Operations
CC:	IACUC Files
Date:	12/28/15
Re:	IACUC Protocol 1522C-KB-Horse-18 Approval

The UNC IACUC has completed a final review of your protocol "*Brainstem Auditory Evoked Response in Old Versus Young Horses*". 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 1522C-KB-Horse-18.

The next annual review will be due before December 28, 2016.

Sincerely,

Laura Martin, Director of Compliance and Operations

APPENDIX B

LETTER OF INFORMED CONSENT

UNIVERSITY OF
NORTHERN
COLORADO

Audiology and Speech-Language Sciences
School of Human Sciences
College of Natural and Health Sciences
University of Northern Colorado
Gunter Hall, Room 1400, Campus Box 140
501 20th Street
Greeley, Colorado 80639

Project Title: BAER differences in old versus young horses

Principal Investigators: Brenna Melvin, B.S. & Kathryn Bright, Ph.D.
Contact Phone Number: (530) 356-4518
Contact E-mail: melv7844@bears.unco.edu

Faculty Advisors: Kathryn Bright, Ph.D. & Tina Stoody, Ph.D.

You have been asked to allow your horse to receive a hearing test as part of a study being conducted at the University of Northern Colorado (UNC). Your horse will receive a Brainstem Auditory Evoked Response (BAER) test.

We will be using very tiny, subdermal needles placed in three (3) different locations on the horse. Lidocaine/Prilocaine (2.5%/2.5%) will be applied to these locations, before placing the electrodes, for numbing of the area. Cashel foam insert earphones will be inserted into the ear canal of the ear being tested, and a click stimulus will be presented.

The test requires the horse to stay relatively still. For horses that exhibit too much movement during testing or show excessive stress/anxiety testing may need to be rescheduled with sedation administered by an attending veterinarian upon your permission. You will receive a report on the state of your horse's auditory health and any recommended follow-up activities. All hearing screenings/assessments will be analyzed and confirmed by two (2) audiologists.

By signing below, you indicate that you understand that your horse's participation is voluntary and that you may withdraw your horse from the test at any time. You also understand that you remain responsible for your horse's health and behavior, and that UNC will not be responsible for injuries to your horse, or injuries or property damage caused by your horse.

HORSE OWNER	**PRINCIPAL INVESTIGATOR**
Signed: _____	Signed: _____
Name: _____	Name: _____
Phone or Email: _____	Date: _____

APPENDIX C

RAW DATA

Participant #	Age	Absolute Latencies (ms)			Interpeak Latencies (ms)			Wave Amplitudes (uV)			Threshold (dB SPL)
		I	III	V	I-III	III-V	I-V	I	III	V	
1	5	2.40	3.55	5.75	1.18	2.17	3.35	0.42	0.27	0.10	60 dB
2	6	2.35	3.58	5.70	1.23	2.12	3.35	0.32	0.19	0.54	70 dB
3	7	2.35	3.60	5.45	1.25	1.85	3.10	0.37	0.07	0.33	80 dB
4	7	2.30	3.50	5.50	1.20	2.00	3.20	0.13	0.08	0.37	70 dB
5	20	2.40	3.55	5.78	1.15	2.23	3.38	0.19	0.27	0.16	80 dB
6	24	2.42	3.42	5.40	1.00	1.98	2.98	0.25	0.49	0.18	60 dB
7	25	2.17	3.85	5.30	1.68	1.45	3.13	0.32	0.37	0.23	80 dB
8	31	2.25	3.58	5.53	1.33	1.95	3.28	0.46	0.09	0.34	60 dB
9	31	2.30	3.33	5.00	1.03	1.67	2.7	0.08	0.64	0.13	90 dB