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Extended High Frequency Brainstem Auditory Evoked Testing in Aging Canines

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EXTENDED HIGH FREQUENCY BRAINSTEM AUDITORY EVOKED RESPONSE TESTING IN AGING CANINES

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ABSTRACT

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Presbycusis, or age-related hearing loss, has a common pattern of high frequency hearing loss that progresses as humans age. It is thought that canines experience a similar pattern of hearing loss as they age. Currently, there is little research on how aging impacts canine hearing acuity. This study was conducted to obtain data to determine if thresholds for the brainstem auditory evoked response (BAER) were different for two types of acoustic stimuli in older dogs. Ten dogs age nine or older were tested but data from two dogs were removed after the initial analysis. Threshold estimations were performed using clicks and 12kHz tonebursts. Stimuli were initially presented at a high intensity level of 92 dB peSPL. Wave V peaks were marked as the intensity decreased until peaks could no longer be identified; this was determined to be threshold. If no response was found at the initial presentation level of 92 dB peSPL, then intensity was increased to 102 dB peSPL. If no peak was identified at 102 dB peSPL, then testing was terminated and a no-response finding was recorded. For data analysis, 112 dB peSPL was used as threshold for the dogs with no responses. The results showed a statistically significant difference in the average thresholds for the click and 12kHz toneburst. The average threshold using clicks was 65.75 dB peSPL while the average threshold using 12kHz tonebursts was 92 dB peSPL. Since responses to clicks are thought to represent hearing sensitivity in the 2-4kHz range and responses to 12kHz tonebursts represent higher frequency responses, the difference in hearing thresholds for a group of older canines might be an indication of a decline

in auditory responses in higher frequency regions of the cochlea as canines age. It is possible hearing loss might be occurring within frequency regions above those assessed by click stimuli, and clinicians might want to consider incorporating high frequency toneburst testing into diagnostic BAER protocols in aging canines. More data on younger canines are needed before conclusions can be made about age-related hearing loss in canines.

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STATEMENT OF THE PROBLEM

Introduction

As human beings age, many changes occur in the auditory system that typically lead to a pattern of hearing loss referred to as presbycusis. Presbycusis is characterized by a downward sloping, high frequency, sensorineural hearing loss in the early stages; however, as it continues to impact the mid and low frequencies, the configuration may flatten out (Lee, 2013; Schuknecht & Gacek, 1993). The range of frequencies normal-hearing adult human beings can perceive is between 20 Hz and 20,000 Hz while the canine range of hearing is between 64 and 44,000Hz (Purves et al., 2011; West, 1985). Presbycusis typically begins in the 10kHz to 16kHz range and can occur as early as age 30 to 39 in humans. However, this high frequency loss is not typically observed during standard audiometric testing that traditionally only evaluates frequencies between 250 and 8,000 Hz in humans. A brainstem auditory evoked response (BAER) test using a click stimulus is most sensitive to hearing loss in the 2-4kHz range in canines, which would also miss a high frequency loss. Hearing loss in humans is thought to progress into the 6-8kHz range as early as age 40 years and by the time human beings reach their 60s, hearing loss that impacts the speech frequencies (.5-4kHz) is common (Yang et al., 2015). Since presbycusis shows a progressive pattern of hearing loss that begins in the high frequencies in both humans and canines (Ter Haar et al., 2008, 2009; Shimada et al., 1997; Yang et al., 2015), it stands to reason that consideration should be given to adapting test procedures to reflect those changes in canines.

Evidence from current research supports the theory that age also impacts canine hearing (Ter Haar et al., 2008, 2009). Ter Haar et al. (2008) found not only that aging impacts canine BAER thresholds but they also found the frequencies most impacted by aging were 8-32kHz. They also found shifts in thresholds starting around age eight in dogs. It was determined that the most significant shift occurred in the oldest group of dogs. These researchers determined these changes could be seen using BAER testing. While threshold shifts can eventually spread to the entire frequency range, the initial shifts were most pronounced in the mid to high frequency ranges like the changes in humans. Ter Haar et al. (2009) compared the cochleae of three young dogs and 10 geriatric dogs. Brainstem auditory evoked response testing was done on the dogs while sedated and after, the 13 dogs were humanely euthanized in order to study auditory systems. The researchers found cochlear lesions in all the geriatric canine cochleae. There was also a reduction in inner and outer hair cells, spiral ganglion density in the basal turn of the cochlea, and stria vascularis. This indicated physiological changes in the canine ear associated with aging. There is a growing body of research regarding canine hearing but it is still unclear what protocols should be put into place when testing older canines.

Untreated hearing loss can cause negative impacts on mental health, cognition, social interactions, and overall quality of life in humans. While hearing loss might not have the same impacts on quality of life for dogs, it could still lead to several problems such as safety and behavioral concerns (Houpt & Beaver, 1981; Scheifele & Clark, 2012; Strain, 1996). Better data about how canine hearing changes over time is needed to determine treatment options. In collecting this data, it is important to utilize an effective protocol that targets the most important information with the least stress to the dogs.

Standard audiometric testing entails presenting acoustic stimuli, usually pure tones, to a person who then responds by raising their hand or pushing a button to indicate a stimulus was heard. Animals cannot participate in this kind of behavioral test without significant time spent training them so effective objective testing is needed to estimate hearing acuity in canines. The brainstem auditory evoked response (BAER) is an objective test, meaning it does not require a behavioral response from the test subject. The BAER is performed by presenting an acoustic stimulus, usually clicks or tone bursts, via an earphone inserted into the animal's ear canal and then using needle electrodes to measure the changes in voltage that occur as the stimulus information is transduced to an electrical signal and carried from one nerve cell/neuron to the next upwards along the auditory nerve and through the brainstem. These voltage changes are plotted in time as a waveform. Because animals are not able to provide consistent behavioral responses to standard audiometric testing in a time-efficient manner, BAER testing is the gold standard for assessing hearing in animals (Ter Haar et al., 2002).

At this time, most BAER research is performed using clicks because they are transient stimuli that produce a more synchronous neural response. A drawback to using clicks is they do not provide frequency specific information. Also, while clicks are broadband stimuli due to the mechanics of the basilar membrane, the response most represents auditory function between 2-4kHZ (Gorga et al., 2006). Because clicks provide minimal high frequency information, they might not be the most efficient stimuli to detect the high frequency loss often characterizing presbycusis, particularly in canines due to their larger range of high frequency hearing.

The aim of this study was to determine whether BAER testing using a high frequency toneburst resulted in different hearing threshold information than BAER testing using click stimuli in older canines.

Research Question and Hypothesis

Q1 Do wave V thresholds differ significantly between a click stimulus and a 12kHz toneburst stimuli in aging canines nine years and older?

H1 Click and 12kHz thresholds will differ significantly in canines (nine years and older) such that12kHz thresholds will be higher than click thresholds.

CHAPTER II

REVIEW OF THE LITERATURE

Anatomy and Physiology of Human Hearing

The ear is divided into three main parts: outer, middle, and inner. The outer ear consists of the pinna or auricle and the external auditory meatus (EAM), which is also referred to as the ear canal. The pinna is a cartilaginous structure that aids in localization, protection of the EAM, and in funneling sound into the EAM.

The ear canal is made up of a cartilaginous and osseous portion; the cartilaginous portion is more lateral while the bony portion is medial. The ear canal serves to move sound down to the middle ear as well as to protect the tympanic membrane and middle ear (Glasscock et al., 1987; Hayes et al., 2013; Miller, 2013). The ear canal contains ceruminous and sebaceous glands that create cerumen or wax which coats the canal and is a form of protection against foreign objects (Glasscock et al., 1987; Lopez de Nava & Lasrado, 2019; Miller, 2013). The s-shape of the canal also serves to protect the tympanic membrane from foreign objects. The ear canal terminates at the tympanic membrane (Glasscock et al., 1987; Hayes et al., 2013; Miller, 2013).

The tympanic membrane is a thin, three-layered barrier between the outer and middle ear. The inner and outer layers are comprised of epithelium cells and the middle layer is a fibrous connective tissue. The tympanic membrane is divided into the pars tensa and pars flaccida. The pars tensa is the stiffer portion of the tympanic membrane containing an abundance of elastin fibers and makes up most of the membrane while the pars flaccida is a loose structure occupying a small upper corner of the tympanic membrane. The tympanic membrane vibrates as it

encounters sound waves, transmitting the acoustic vibrations of sound mechanically along the ossicular chain of the middle ear (Glasscock et al., 1987; Hayes et al., 2013; Miller, 2013).

The distal border of the middle ear is formed by the tympanic membrane and the proximal aspect is defined by the cochlear promontory—a bony prominence formed by the basilar turn of the cochlea. There are two membrane-covered openings on the promontory: the oval window and the round window. The middle ear is an air-filled cavity that resides between these two borders and contains the ossicular chain—a series of three tiny bones named the malleus, incus, and stapes (Glasscock et al., 1987; Hayes et al., 2013; Miller, 2013). The manubrium, or arm, of the malleus attaches the ossicular chain to the tympanic membrane and can be visible with otoscopic examination. The incus connects the malleus to the stapes. The footplate of the stapes rests over the opening of the oval window. As the footplate of the stapes moves in and out of the oval window with the vibratory motion of sound passed down the ossicular chain, it transmits that energy to the inner ear (Glasscock et al., 1987; Hayes et al., 2013).

The inner ear consists of two parts: the cochlea and the vestibular system. The vestibular system is important for balance and will not be discussed further. The cochlea is the snail shaped organ responsible for transduction of sound into an electrochemical signal that is sent to the brain (Eggermont, 2019; Glasscock et al., 1987; Hayes et al., 2013; Hopkins, 2015; Nam & Fettiplace, 2012; Purves et al., 2011). In humans, the cochlea is approximately 55 mm in length and it turns 2.5 times before reaching the apex or top. The base, or basal end, is wider and the apex is narrower. Three fluid-filled chambers—the scala tympani, scala vestibuli, and scala media—are present in the cochlea. Perilymph resides in the scala tympani and scala vestibuli, while endolymph resides in the scala media. This inner chamber is referred to as the cochlear duct and

it houses the organ of Corti. The basilar membrane forms the base for the organ of Corti and separates the cochlear duct from the scala tympani while the Reissner's membrane forms the roof of the cochlear duct and separates it from the scala vestibuli. Within the organ of Corti lie sensory cells known as hair cells. There are two types of hair cells: the outer hair cells (OHC) and the inner hair cells (IHC). There are typically three rows of OHCs and one row of IHCs. Fibers from the auditory nerve innervate the base of both types of hair cells. Most of the fibers innervating the OHCs are efferent or come from the central nervous system while the IHCs are mostly innervated by afferent nerve fibers. Ninety-five percent of all afferent fibers in the auditory system that go to the brain come from the IHCs (Eggermont, 2019; Glasscock et al., 1987; Hayes et al., 2013; Hopkins, 2015; Musiek & Baran, 2007; Nam & Fettiplace, 2012; Purves et al., 2011). The efferent system is still a bit of a mystery but it is believed it works with the afferent system to create a feedback loop within the auditory system (Musiek & Baran, 2007). Efferent fibers have larger, vesiculated endings that come from the efferent neurons of the olivocochlear bundle. The endings of the efferent fibers have direct contact with the OHCs but the efferent fibers terminate on the afferent fibers coming from the IHCs instead of having direct contact like with the OHCs (Musiek & Baran, 2007). On top of the hair cells are stereocilia, which are connected by tip links (Hopkins, 2015; Musiek & Baran, 2007). The stereocilia on the OHCs connect the hair cells to the tectorial membrane; there are typically three rows of stereocilia on the OHCs that form a "W" shape. There are more OHC stereocilia at the base of the cochlea than there are at the apex (Musiek & Baran, 2007).

As the footplate of the stapes moves from the sound vibrations, it displaces the perilymph in the scala vestibuli. When the perilymph is displaced, it causes the basilar membrane to move. The basilar membrane is displaced by the sound wave and each frequency has a specific area

along the membrane that is most sensitive to that frequency. For example, higher frequencies displace the basilar membrane more at the base of the cochlea while lower frequencies cause maximum displacement closer to the apex (Eggermont, 2019). This movement of the basilar membrane causes the hair cells to move as well. The OHCs enhance the movement of the basilar membrane as well as amplify the vibrations to help the IHCs detect the sound (Eggermont, 2019; Glasscock et al., 1987; Hayes et al., 2013; Hopkins, 2015; Nam & Fettiplace, 2012; Purves et al., 2011). The IHC cells responsible for converting a mechanical signal into an electrical signal. As the basilar membrane and IHCs move, the stereocilia on the IHCs bends. The tip links, which connect the stereocilia, stretch and open ion channels, allowing potassium to enter the cell and cause depolarization. The depolarization increases the chance of an action potential occurring, which will then send the signal up the afferent auditory nerve pathway to the central nervous system (Gelfand, 2007; Hopkins, 2015; Musiek & Baran, 2007).

The stereocilia are stiff; however, that stiffness changes with polarization. When the cell is depolarized, the stiffness decreases, and stiffness increases as the cell becomes hyperpolarized. The movement of the stereocilia causes the OHC to open and close, which in turn sends excitatory and inhibitory signals up the auditory nerve. The hair cells and stereocilia are very tiny and fragile. Noise, medications, aging, and otic diseases can cause damage to the stereocilia. Damaged stereocilia cannot be repaired, leading to permanent hearing loss (Gelfand, 2007; Glasscock et al., 1987; Hayes et al., 2013; Miller, 2013; Musiek & Baran, 2007).

The efferent and afferent nerve fibers of the cochlea attach to the auditory nerve (AN). Information from the cochlea is coded and passed up through the AN to the central auditory system (CANS). The AN is approximately 22-26mm in length and has around 30,000 nerve fibers. Two types of fibers in the AN are known as Type I and Type II fibers. Type I fibers

connect to the IHCs of the cochlea; approximately 90% of the fibers in the AN are Type I. Type II fibers connect to the OHCs and make up the remaining 10% of fibers in the AN. Multiple AN fibers can innervate 1 IHC, while 1 AN fiber can innervate multiple OHCs. The AN runs from the terminal ends of the hair cells and through different openings in the bone such as the habenula perforata and Rosenthal's canal. The fibers then form the modiolus and pass through another opening known as the internal auditory meatus. After that, the fibers enter the cerebellar pontine angle before leading into the first central auditory junction, which is the cochlear nucleus.

The cochlear nucleus is the beginning of the CANS. The cochlear nucleus has three major parts: dorsal, posterior ventral, and anterior ventral cochlear nucleus. The fibers from the auditory nerve project into the cochlear nucleus between the anterior ventral and posterior ventral nuclei. The pathway from the cochlear nuclei leads to three main routes to the ventral, dorsal, and intermediate stria. The ventral stria, also known as the trapezoidal body tract, either sends information up to the lateral lemniscus or the fibers decussate and synapse on the contralateral nuclei of the superior olivary complex, while the dorsal pathway forms the intermediate acoustic stria, which leads to the contralateral lateral lemniscus. The cochlear nucleus also has fibers that travel to the superior olivary complex and the inferior colliculus. From there, the signal travels to the medial geniculate body up to the auditory cortex (Gelfand, 2007; Musiek & Baran, 2007).

Aging can lead to damage in the inner ear and the central auditory nervous system. The loss of sensory hair cells, metabolic cells, and cranial nerve VIII neurons can cause hearing loss that typically manifests as a high frequency sensorineural hearing loss (Frisina, 2001). These changes can often be observed via changes in both electrophysiological and behavioral tests.

Presbycusis

Age-related hearing loss, or presbycusis, occurs commonly in adults 65 or older. About one in three adults between 65 and 75 have hearing loss and increases to one in two for adults over 75 years old (National Institute on Deafness and Other Communication Disorders, 2018). It is typically characterized by a gradual, bilateral, sloping, sensorineural hearing loss. Decreased speech understanding is also commonly observed with hearing loss that is thought to be age related (Lee, 2013; National Institute on Deafness and Other Communication Disorders, 2018). No medical intervention can correct presbycusis at this time (Gates & Mills, 2005; Lee, 2013). There are several classifications of presbycusis: sensory, neural, strial or metabolic, cochlear conductive, mixed, and indeterminate (Lee, 2013; Schuknecht & Gacek, 1993).

Each type of presbycusis has unique characteristics and origins. The outer hair cells in the organ of Corti begin to deteriorate in sensory types of presbycusis. Animal and human studies showed this deterioration around the basal turn of the cochlea. This damage resulted in a symmetrical high frequency hearing loss. Sensory presbycusis made up 5% or less of presbycusis cases (Lee, 2013; Schuknecht & Gacek, 1993).

In cases of neural presbycusis, the damage occurs in the nerves. To be considered neural presbycusis, 50% or more of the cochlear neurons will show degeneration. Afferent nerve loss leads to decreased speech discrimination when 50% of neurons are damaged while a shift in hearing threshold takes about 90% neuronal loss. Clinically, a moderate sloping hearing loss with disproportionately worse speech discrimination is what defines this loss (Lee, 2013; Schuknecht & Gacek, 1993).

The stria vascularis is impacted in the strial or metabolic presbycusis and the entire frequency range can be impacted in this type of hearing loss (Lee, 2013; Schuknecht & Gacek,

1993). The stria vascularis is an important structure that is responsible for maintaining the ion balance in the endolymph in the scala media (Liu et al., 2016). Metabolic presbycusis is thought to be the main type of presbycusis (Lee, 2013; Schuknecht & Gacek, 1993).

While there is still confusion about the cochlear conductive form of presbycusis, it is thought to be caused by a stiffening of the basal end of the cochlea, which is thought to worsen with age. Unlike other forms of presbycusis, cochlear conductive hearing loss can result in a low frequency loss with little or no impact to speech understanding (Lee, 2013; Schuknecht & Gacek, 1993). Due to the differences from other types of presbycusis, there is still some uncertainty if this type of hearing loss should be classified as presbycusis (Lee, 2013).

Mixed presbycusis is a combination of the previously discussed types of age-related hearing loss. Damage is seen in the outer hair cells, stria vascularis, and cochlear neurons (Lee, 2013; Schuknecht & Gacek, 1993). Mixed presbycusis presents a sloping hearing loss like the sensory and neural type; however, there is often a recovery in the high frequencies. The damage to the outer hair cells leads to the loss of high frequencies but the addition of strial loss can also cause a low frequency hearing loss (Lee, 2013).

Indeterminate is the last type of presbycusis and there is no clear pattern of hearing loss or where the damage is occurring. While it is unclear where the damage occurs, it is thought to be in the tip links of the stereocilia or due to a central hearing impairment. Indeterminate presbycusis is thought to account for as much as 25% of presbycusis cases (Lee, 2013; Schuknecht & Gacek, 1993). Presbycusis in all its forms is one of the more commonly seen conditions by audiologists and it is important to know how this condition will impact testing such as auditory brainstem responses.

Presbycusis can lead to changes in the brain that often shows clinically as increased difficulty in noise. The ability to understand and process speech often declines slightly as humans age; however, presbycusis can cause additional trouble with speech in the elderly. Wong et al. (2009) looked at single word understanding in a young group and an elderly group. Single words were given in quiet and two multi-talker babble conditions. One condition had a signal to noise ratio (SNR) of +20dB with the signal being louder than the babble and one condition had a -5dB SNR, meaning the signal was softer than the babble noise. Both groups performed well in quiet and with the +20dB SNR; however, the elderly group performed worse in the -5dB SNR multi-babble condition. In addition to the behavioral results, each participant was given an fMRI to measure activity in the brain in each condition. The older participants showed reduced activity in the right superior temporal region while having an increase in activity in the posterior parietal and prefrontal areas in all conditions compared to the young subjects. The young group showed an increase in activation in the superior temporal regions that corresponded with the level of noise (i.e., as level of noise increased so did activity); however, they did not show increases in activity in the posterior parietal and prefrontal areas as did the older group. This showed how the brain compensated for a decrease in function due to aging. Wong et al. suggested their results supported the idea that changes to the auditory cortex could precede peripheral hearing changes; however, the compensations near the prefrontal and parietal lobes could lead to these changes not showing up on behavioral tests early during the degenerative process (Vercammen et al., 2018; Wong et al., 2009).

A study done by Lee et al. (2005) demonstrated how thresholds changed over time using both standard audiometric testing and extended high frequency pure tone testing. In their study, 188 adults between the ages of 60 and 81 years old had their hearing tested multiple times across

a period of 3 to 11 years (mean time period of 6.40 years). Each participant was tested a minimum of two times and a maximum of 21 times with an average of 9.81 test sessions. The conventional audiometric range consisting of pure tones between 250Hz and 8kHz was tested at each appointment, while extended high frequencies consisting of pure tones from 9-19kHz were tested at the initial intake and then every two to three years. The researchers used the slope of linear regression to determine the rate of change in hearing thresholds over time. The overall rate of change in threshold was approximately 1dB per year with a faster rate in older subjects. The rate of change was larger in higher frequencies and 12kHz had the quickest rate of change at approximately 1.23dB per year. This study in humans showed that thresholds in the high frequencies were more likely to change at a faster rate than lower frequencies (Lee et al., 2005).

Evaluation of Auditory Function in Humans

Behavioral Testing

Behavioral testing requires the listener to respond in some manner (conventional responses include pushing a button, raising their hand, etc.) when a stimulus is presented. Clinicians modify the testing procedure for children and other patient populations who are unable to reliably respond to conventional audiometry (Northern & Downs, 2014). Canines are not able to be assessed behaviorally without special training so behavioral testing was not discussed further.

Objective Testing

When reliable behavioral responses cannot be obtained, objective tests can be used instead. Objective tests are commonly used in audiology with infants, young children, and other difficult-to-test populations. They allow audiologists to assess the integrity of the auditory system but it is important to note they do not test hearing. These objective tests are unable to

determine if the patient understands and processes the sound but they do show whether the part of the system they are assessing is functioning properly. Some objective tests can be used to estimate hearing sensitivity. Common objective tests are otoacoustic emissions (OAEs) and auditory evoked potentials such as the auditory brainstem response (ABR). Otoacoustic emissions are useful in assessing the health of the outer hair cells in the cochlea in humans and animals. Otoacoustic emissions are quick and could be part of the hearing assessment for canines; however, they were not used in this study. Some limitations with OAEs are they do not indicate how severe the hearing loss is and cannot determine threshold, which was the point of this study. For these reasons, no further discussion of OAEs was necessary for this study and remaining discussion of objective testing will focus on auditory evoked responses.

Auditory Brainstem Response

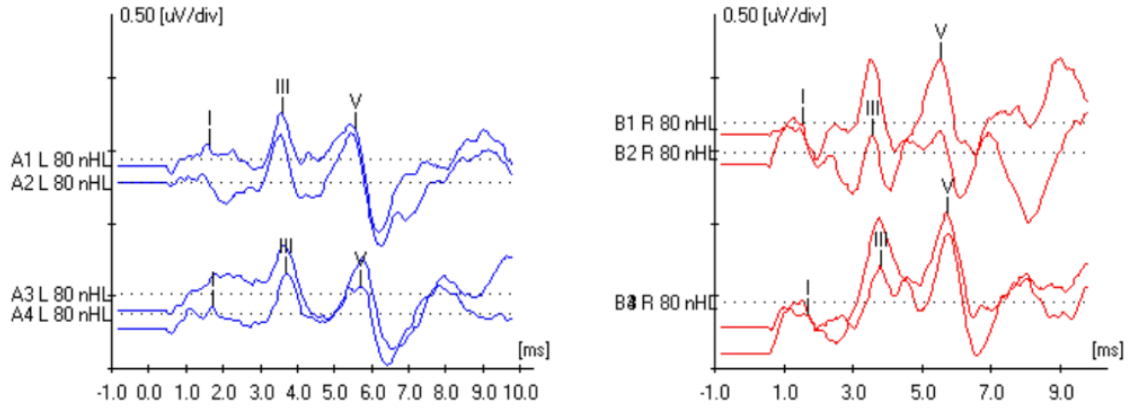
The ABR is an objective, electrophysiologic test that allows audiologists and related professionals to estimate an individual's hearing thresholds and assess the integrity of the auditory system. An ABR does not require a behavioral response from the patient to obtain reliable results; however, a trained professional is required to interpret the findings (American Electroencephalographic Society, 1984). The ABR is an early electrophysiological test as it occurs within 10 milliseconds (ms)following the presentation of the stimulus. The responses within the central auditory nervous system are time locked to the presentation of the stimulus so typical patients' responses should be occurring within specific time frames in normal hearing individuals. The sudden onset of the signal causes the neurons to fire synchronously, causing the time locked response (Paulraj et al., 2015).

An ABR typically has five to seven positive peaks within a 10ms time window. In the United States, positive polarity responses are recorded as upward facing peaks while negative

polarity responses are recorded as downward troughs. The peaks are typically labeled using Roman numerals (Jewett & Williston, 1971). While not a perfect one-to-one relationship, each ABR wave is thought to be generated from a different area of the central auditory system. Wave 1 arises from the auditory nerve before it leaves the cochlea while Wave II comes from the auditory nerve after it exits the cochlea. Wave III arises in response to stimuli at the level of the brainstem (Atcherson, 2012; Eggermont, 2019). Wave III arises from the cochlear nucleus; wave IV is from a site near the lateral lemniscus. Wave V is between the lateral lemniscus and inferior colliculus (Atcherson, 2012; Eggermont, 2019). Auditory brainstem responses are typically used as a neurodiagnostic test (see Figure 1) or to estimate a patient's threshold (see Figure 2).

Figure 1

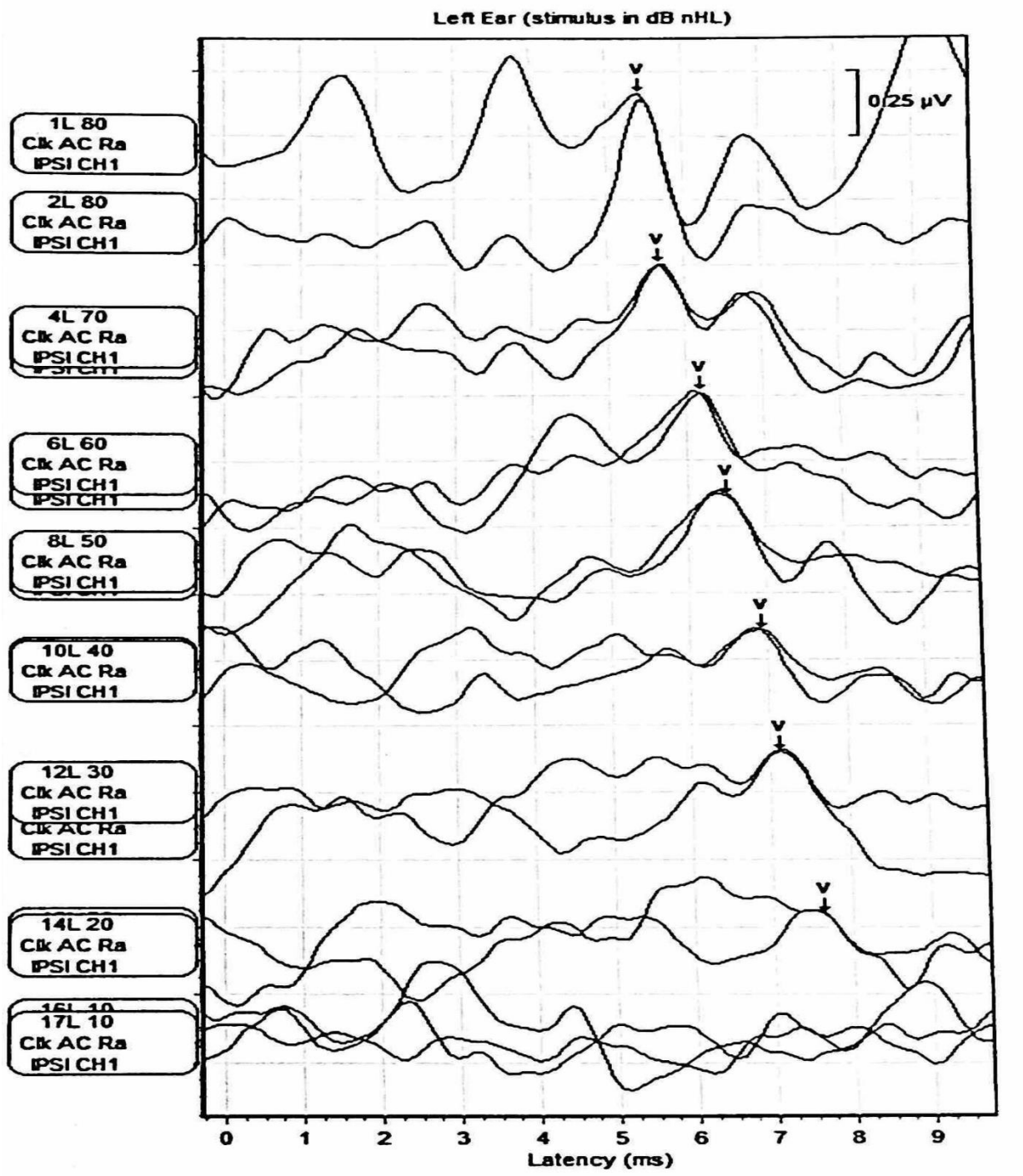
Neurodiagnostic Auditory Brainstem Response Example



Note. Results of a neurodiagnostic ABR using a click stimulus were taken from the researcher of this study, a 25-year-old normal hearing female at the time of testing. The left ear waveforms are displayed in blue, while the right ear waveforms are displayed in red. At 80 dB nHL Waves I, III, and V are identifiable and repeatable. While not listed, the absolute and interpeak latencies are within normal limits

Figure 2

Threshold Auditory Brainstem Response Example



Note. Threshold ABR search done using click stimulus on 27-year-old female with normal behavioral thresholds. On the left of the waveform is information on which ear was tested, intensity levels, and type of stimulus. The ABR thresholds were within 20dB of the behavioral thresholds.

Neurodiagnostic ABRs can be used to assess the integrity of the auditory system. In a neurodiagnostic ABR, a high intensity stimulus is used to bring about the best wave morphology or appearance. Wave I should occur around 1.5 millisecond and each wave after should appear approximately one millisecond later. The time each wave appears is referred to as the absolute peak latency. Wave V is typically the most clear and consistent wave; it is the most often evaluated, particularly when searching for thresholds. Waves I and III are also typically visible at higher intensities while Waves II and IV are the most likely to be absent or difficult to distinguish from other waves.

Interpeak latency is a term used when looking at the timing between waves. Interpeak latencies are typically examined between Waves I, III, and V. Delays in the interpeak latencies could indicate a present pathology. In individuals without hearing loss, absolute latencies should occur around the same time. Delays in absolute latencies could also indicate a possible hearing loss (Hall, 2007). Changes that occur to the ABR in the presence of hearing loss could help determine the type of hearing loss. A conductive pathology typically leads to a delay of all absolute latencies with the interpeak latencies being unaffected. A sensorineural hearing loss could present several different ways. Some sensorineural losses might have no impact on the ABR, Wave V could be delayed, or the waveform could be absent (Atcherson, 2012). A retrocochlear pathology is most likely to have a normal Wave I latency but the interpeak latencies for Waves I-V and III-V are typically elongated due to delayed or absent Waves III and V. A retrocochlear pathology is likely to have poor morphology as well (Śliwińska-kowalska, 2015).

Audiologists can estimate a patient's behavioral threshold by following Wave V as they systematically decrease the stimulus intensity. Latencies increase and morphology worsens as

intensity of the stimulus decreases; the poor morphology caused by lower intensity makes the waves unidentifiable except for Wave V until it nears threshold. The lowest intensity with a repeatable Wave V is the threshold. The ABR typically estimates behavioral thresholds within 5-20dB of the patient's Wave V thresholds (Abbas et al., 1985; Boettcher, 2002; Glasscock et al., 1987).

Equipment and Recording

To record an ABR, multiple pieces of specialized equipment and supplies are needed such as stimulus generators and amplifiers which need to be utilized using a computer with specialized software as well as transducers that send the signal from the computer to the patient's ear. A stimulus generator is used to create the signal. It also can control the polarity of the signal (Glasscock et al., 1987). Another piece of equipment needed is a differential amplifier. The small amplitude of the ABR waveforms could make it difficult to see a response over biological and environmental noise so an amplifier is used to help make the evoked response of interest larger. Electrodes, which attach to different locations around the head and neck, are used to detect electrical changes along the brainstem as the sound stimulus is delivered to the ear (Eggermont, 2019; Maltby, 2016; Martin & McFerran, 2017; Ter Haar et al., 2002). The 10-20 system designates specific sites on the head for electrode placement (Atcherson & Stoody, 2012; Homan, 1988). A minimum of three electrodes are needed to record an ABR: a positive or non-inverting electrode, a reference or inverting electrode, and a ground electrode. Several electrode placements can be used to record an ABR but typically the non-inverting electrode is high on the scalp (top of the forehead or the vertex of the head) while the reference and ground electrodes are placed on the earlobes or mastoids. The reference electrode is placed by the test ear and often the ground electrode is on the non-test ear (Atcherson & Stoody, 2012; Glasscock et al., 1987;

Hall, 2007). While the ground electrode can be placed elsewhere, this arrangement could be easier for the tester to switch ears (Hall, 2007). Appropriate electrode placement could be helpful in reducing noise and leads to better test-retest reliability. In addition, amplitude varies based on where the active electrode is placed (Atcherson & Stoody, 2012; Glasscock et al., 1987).

One method to reduce noise and increase the signal is common mode rejection. The concept of common mode rejection is to take parts of the signal that are the same and reject those while amplifying the difference between the recording sites. Common mode rejection requires a differential amplifier that takes both signals and cancels out the like noise while amplifying the signal that is different between the two sites. The differential amplifier is unable to employ subtraction so one signal is inverted and the two signals are added together. For common mode rejection to effectively amplify the signal, electrodes must be placed far enough away to find a difference between the signals (Abbas et al., 1985; Atcherson & Stoody, 2012; Crumly, 2011; Glasscock et al., 1987; Oshrin & Terrio, 1989). According to Atcherson and Stoody (2012), optimal amplification is achieved at opposite ends of a dipole. A dipole is best thought of as the separation of charges. The positive and negative charges are separated at different ends of the neuron which creates a dipole (Atcherson & Shoemaker, 2012).

Signal averaging is another commonly used method that improves the signal to noise ratio (SNR) to draw out the signal of interest. The stimulus is presented many times and then the response is averaged. Since the response is time locked due to the rapid onset of the stimulus, the response should be similar in each run while noise is random and is averaged out (Abbas et al., 1985; Glasscock et al., 1987; Hall, 2007).

There are several different types of transducers but foam inserts are the most common. Insert earphones can reduce stimulus-related artifact. For long periods of time, inserts are

generally more comfortable than other types of transducers and as they are not reused, inserts are more sanitary (Atcherson & Stoody, 2012). Inserts help to create a physical and temporal separation between the ear and the transducer box. This space creates a delay of about .8msec. This is important as it prevents stimulus artifact from interfering with the ABR response. Inserts also have a greater interaural attenuation. Over-ear headphones and bone oscillators are additional transducers and soundfield could also be used to stimulate the ear (Atcherson, 2012; Atcherson & Stoody, 2012).

Filters are put in place in the computer's ABR software to reduce artifact and increase the SNR. Filter settings are determined by the spectral or frequency composition of the auditory evoked potential (AEP). Filters today are typically already established based on known spectral compositions of various AEPs (Atcherson & Stoody, 2012). High pass filters reduce low frequency noise, such as noise from electronic devices, and allow higher frequencies to pass. A low pass filter reduces high frequency noise while letting lower frequencies pass. Frequencies in between these filters are amplified. Low pass filters are typically set at 1500 or 3000Hz and high pass filters are often set at 100 or 150Hz (Hall, 2007). Auditory evoked potential testing typically involves the use of a band-pass filter that incorporates both high pass and low pass filters, allowing only a set of frequencies between the two filter cutoffs through.

Stimulus Effects

Another important factor to the ABR is the stimulus. Transient stimuli are commonly used to elicit an ABR. Clicks and tonebursts are the two most used stimuli, although there are several other types. Click stimuli are used to assess the integrity of the auditory system as a whole and could give a general idea if hearing thresholds are within or close to normal limits. However, a click is most effective at identifying hearing loss between 2k and 4kHz. This occurs

because the spectral energy of the acoustic click has peaks around 2kHz and 4kHz because of how sound waves travel up the cochlea. The traveling wave moves along the basilar membrane and stimulates the base of the cochlea first, meaning the high frequencies are stimulated first. The apical end is stimulated shortly after. Therefore, the majority of the fibers that fire are from the basal and mid ranges (Atcherson, 2012; Gorga et al., 2006). This makes the click ideal at identifying losses around the mid frequencies but it could miss low or higher frequency losses. Instead, tone bursts are commonly used when looking for frequency-specific information (Abbas et al., 1985; Atcherson, 2012; Dagna et al., 2014; Ter Haar et al., 2002). Click stimuli are the most transient and produce a robust response but might not be frequency specific enough to estimate hearing sensitivity across individual frequencies. While click stimuli cannot provide clinicians with frequency- specific information, the toneburst is another stimulus that could be used. A toneburst is a short-duration sinusoid with a quick onset and is used when looking to find frequency-specific thresholds. While clicks have a near instant onset, tonebursts use a gating function to ramp the sound up and off to reduce spectral splatter. This helps make the response more frequency specific. Tonebursts typically have smaller amplitudes than clicks, which could impact morphology. Latencies can differ between tonebursts and clicks with tonebursts often having longer latencies than clicks (Atcherson, 2012; Atcherson & Brueggeman, 2012).

Marttila and Karikoski (2006) performed a study to assess how well a click-evoked ABR could predict behavioral pure tone thresholds. They also examined hearing levels in individuals who had no ABR waveforms with a repeatable Wave V; these tests were done annually over a period of seven years. Eighty-five children with hearing impairments were included in the study. The children ranged in age from .3 to 12.7 years old at the time of the initial assessments. Their ages ranged from 7.3 to 19.7 years at the time of the final assessments. They had all been

referred for hearing evaluations and fit with hearing aids prior to the study. Children with progressive loss, defined as a 10dB change at three frequencies in sequential audiograms, were excluded from the study. Records from previous evaluations and follow-ups were reviewed for each child in the study. Due to the age of the participant during the initial intake, there was an average gap of 5.4 years between the initial ABR and reliable, behavioral responses. All children in the study had otoscopy and tympanometry performed on them to rule out middle ear pathologies. All the ABRs were done following a standardized clinic procedure that used an alternating polarity, a repetition rate of 11.4 clicks/sec, and 2,000 sweeps. The low and high pass filters were set at 3,000 Hz and 150 Hz. Threshold was defined as the lowest stimulation level with a present and repeatable Wave V. All children were sleeping or sedated with chloral hydrate during testing. While the audiologists doing the testing usually attempted to get behavioral results while using over-the-ear headphones, some of the children were tested using soundfield if the audiologists felt the headphones could not be used reliably. Behavioral thresholds were found from .25kHz to 8kHz. The researchers found a visible Wave V in at least one ear in 48.2% of the subjects. The correlation coefficient between the ABR thresholds and pure tone behavioral thresholds was .62 and $p < .01$, which was a significant finding. Of the children with absent ABRs, 66% of them had some residual hearing on their audiograms obtained behaviorally. This indicated the ABR could estimate the pure tone threshold in many cases; however, the researchers found the ABR did tend to overestimate the degree of hearing loss in more moderate and severe hearing losses. They also determined the ABR was most sensitive to hearing between 2kHz and 4kHz. They stated the ABRs could be used to estimate hearing thresholds but cautioned to use with care when estimating more severe hearing losses (Marttila & Karikoski, 2006).

Gorga et al. (2006) went a step further and examined thresholds using a click as well as tonebursts to determine how well the ABR predicted behavioral thresholds. The authors did a retrospective review using medical records from patients. All participants were under the age of 20 at the time of testing and had been referred for a hearing evaluation and ABR; these results were analyzed later for the study. All patients had been tested with ABR using a click and different tonebursts; in addition, behavioral thresholds were obtained prior to the study. If behavioral thresholds could not be obtained, the data were not included in the study. Clicks and 250Hz tonebursts were used and if time permitted, then 1k, 2k, and 4kHz tonebursts were also tested. By the end of the study, the authors had collected data from 140 ears (77 subjects). Patients with auditory neuropathy were excluded from the study. Another condition of the study was consistent middle ear results for each testing session. For example, if a Type A tympanogram was recorded at the first session, a Type A tympanogram needed to be present at each consecutive testing session; data from children who had inconsistencies in the middle ear measures were excluded. The authors found the click ABR had its benefits such as a more robust response when compared to other types of stimuli such as a toneburst, ability to help determine certain disorders such as auditory neuropathy, and it was a relatively quick test. Like the Marttila and Karikoski (2006) study, Gorga et al. found the click could overestimate thresholds and was best at predicting hearing between 2k and 4kHz. However, Gorga et al. found the ABR could underestimate the behavioral thresholds in participants with hearing loss and overestimate behavioral thresholds in individuals with normal hearing sensitivity. The correlation coefficients showed a significant correlation between ABR thresholds and behavioral thresholds for both the click and the 250Hz toneburst. The authors suggested using 250 to get the most information about low frequencies with the least amount of time but they added that the close correlation

between ABR and behavioral thresholds would apply to other toneburst frequencies (Gorga et al., 2006).

Clicks are a good stimulus for getting a general picture about the level of hearing in a subject; however, they could over or underestimate hearing loss and were less accurate at predicting hearing loss at frequencies outside of the 2k to 4kHz frequency range. Using different toneburst stimuli could help give more frequency-specific information as well as give a little information about the configuration of the hearing loss. However, if time is a factor, then clicks are likely to give the most information in the shortest period.

Intensity of the signal is an important aspect to consider. The unit of measurement used for intensity is dB normal hearing level or dB nHL. To calibrate dB nHL, behavioral thresholds are obtained to the click or tone burst thresholds in normal hearing individuals. Threshold is set at 0dB based on the group of normal listeners. A louder stimulus would provide better morphology, higher amplitude, and shorter latencies. As intensity decreased, latency would increase. A general rule was that as intensity decreased by 10dB, Wave V absolute latency was increased by .5ms. The latencies could vary a bit with decreased intensity but the rule of thumb was useful for identifying where Wave V might appear (Abbas et al., 1985; Hall, 2007).

Stimulus rate is an important factor in obtaining an ABR. Slower stimulation rates provide clearer morphology, increased amplitude, and shorter latencies. However, a slower stimulation rate limits the amount of information collected. Stimulation rates that are too fast would typically result in waveforms that have poorer morphology, which could make peak picking difficult. Finding the middle ground between too fast and too slow is important. It is recommended to present the stimulus faster than 20 clicks per second but slower than 40 clicks per second using odd and decimal numbers (Atcherson, 2012). In humans, a recommended

stimulation rate for a neurodiagnostic ABR is lower (often about 10-20 clicks per second) to provide an opportunity for optimum waveform morphology. However, threshold searches could allow for faster stimulation rates (often between 30-40 clicks/second) because a bit of morphology could be sacrificed while still being able to visualize the response (Atcherson, 2012; Glasscock et al., 1987; Hall, 2007). It was also recommended to pick a stimulation rate that was not divisible by two to avoid multiples of the 60Hz cycle that come from electrical sources (Atcherson, 2012).

Polarity describes the movement of the pressure change caused by a sound wave. Due to the phase of the waveform, rarefaction polarity causes movement in the negative direction and causes the stapes to move outward and the organ of Corti to move upward. Condensation polarity is in a different phase of the waveform so it moves in the positive direction and has the opposite effect on the movement of the stapes and organ of Corti. Alternating polarity moves between condensation and rarefaction polarity (de Lima et al., 2008; Hall, 2007). Alternating polarity is good at reducing stimulus artifact that could be seen in low frequency tone bursts. Rarefaction and condensation polarities could cause variation in ABR responses because the phases have different timing; however, this has been found to be clinically insignificant. Some researchers have recommended using rarefaction while others recommended using condensation. However, it is most important to pick a polarity and use it consistently to avoid polarity effects (Coats & Martin, 1977; Glasscock et al., 1987; Schwartz & Berry, 1985).

Patient Factors

Sex

Patient factors are unique to each patient and can have impacts on ABR results. Sex of a patient could impact ABR results in adults. Waves III and V often have larger amplitudes and

shorter latencies in females, possibly due to physiological differences in the length of the basilar membrane and auditory pathway (Glasscock et al., 1987; Picton et al., 1981). Jerger and Hall (1980) found females with and without hearing loss had earlier latencies than males. They found the female latencies to be about .2msec earlier than the male latencies. Glasscock et al. (1987) reviewed their records of 20 males and 20 females under 30 years of age. They found male patients' latencies were on average .15msec longer than the female patients. Other issues such as age and hearing loss could impact ABR results as well.

Age

The ABR can change throughout a lifespan and those changes are often very pronounced during the maturation process. Infants and young children have later latencies than adults because the auditory and central nervous systems are still developing. Fria and Doyle (1984) examined the maturation of the ABR. They hypothesized that maturation occurred in two stages. The first stage was a rapid decrease in wave latency while the second stage was a more gradual decrease in latency particularly for Wave V. In this retrospective cross-sectional analysis, the records of 466 patients were reviewed. All the records showed the latencies of Waves I, III, and V. The results were found at an intensity level of 60dB nHL. All the responses were from normal hearing individuals; to determine they were normal hearing, Wave V had to be visible at 20dB nHL. There were eight groups in the study: a newborn group, 6-8 weeks, 3 months, 6 months, 12 months, 24 months, 36 months, and an adult group (18-35 years). The same system was used to test all the individuals to rule out any differences caused by different ABR systems and 21.1 clicks/second was the stimulation rate for all the individuals in the study. The mean latencies for each age group were compared and differences noted. The researchers found there was a rapid decrease in latency around 8-10 weeks postpartum and more gradual decreases in latency until

about 36 months. The authors stated these findings indicated greater changes in peripheral and central development in the 8-10 weeks postpartum and believed the more gradual changes in ABR latency reflected central development (Fria & Doyle, 1984).

Sharma et al. (2016) also showed changes in the auditory system due to maturation; however, their study showed maturation occurred for much longer. This study contained 80 participants; the participants ranged from newborn to 12 years of age. The participants were divided equally into eight groups: 0-6 months, 7-12 months, 13-24 months, 25-36 months, 37-48 months, 49-60 months, 61-84 months, and 85-144 months. All the participants had normal hearing with no history of hearing loss, ear infections, or neurological disorders that would impact the testing. Otoscopy, OAEs, and behavioral audiometry were performed on all participants to rule out any unidentified hearing losses. A click stimulus was used with a stimulation rate of 21.1 clicks per second. The testing was done at 30B nHL with 2,000 presentations. The authors examined Wave V of each subject and found the latency of Wave V rapidly decreased until about three years old. A decrease in latency was observed between the ages of one and two years, which the authors attributed to axon growth. They also found a slight decrease in latency continued to occur until the age of 12 when they stated maturation was complete (Sharma et al., 2016). Changes in ABR latencies occurred most dramatically in the first three years of life in humans with some additional decreases in latencies carrying on until around 12 years old (Sharma et al., 2016). While maturation could have dramatic effects on the auditory system and by extension the ABR, additional changes were also noted in both the auditory system and the ABR as adults aged into their senior years.

Konrad-Martin et al. (2012) examined the effects of aging on ABR results. This study contained 131 veterans between the ages of 26 and 71. Participants were divided into groups by

age as follows: <40 years, 40-49 years, 50-59 years, 60-69 years, and 70+ years old. Exclusion criteria included individuals wearing hearing aids, those who had cancer or received cancer treatments, multiple sclerosis or other neurological diseases, dementia, communication difficulties, or individuals with hearing loss in both ears. Participants could not have thresholds higher than 40dB at 2,000 Hz and 75 dB at 4,000Hz in at least one ear. Those with conductive losses were not included in this study. Participants were also broken up into better and worse hearing ears to examine the impact of hearing loss as well as age. Three different stimulus rates were used: 11, 51, and 71 clicks/second. The authors also looked at the impact of stimulation rate. Both condensation and rarefaction polarity were used. The results of the study showed age did diminish the amplitude of an ABR independent of hearing loss. Waves I and III showed reduced amplitude for the 45 and 55 age group but did not show reduced amplification for the 65 years + age group. Wave V was not impacted by stimulation rate. The latencies of Waves III and V also appeared to be affected by age. The average Wave III latency was between 3.94 and 3.96msec for the participants under 60 years old; however, the average latency for the 60-69 years old group shifted to an average time of 4.06msec and the 70+ group had a latency of 4.11msec. The interpeak latency of Waves I and V was not impacted by aging. The authors hypothesized there would be a reduction in neural synchrony or a decrease in the amount of ABR generators and delayed latencies might be due to changes in the auditory nerve (Konrad-Martin et al., 2012).

Rosenhall et al. (1985) conducted a study to look at how aging impacted the auditory brainstem response. In this study, there were 268 participants whose ages varied from 5-75 years old. Participants were divided into one of six age groups: 5-14, 15-24, 25-34, 35-44, 45-54, and 55-75. All the participants had thresholds better than 20 dB from 125Hz to 2,000Hz and

thresholds better than 35dB from 4,000 to 8,000Hz. The researchers used click stimuli with alternating polarity. The stimulation rate was 25 clicks per second and were presented at 80dB HL (115dB peSPL). The authors looked for all waves but they stated that Waves I, III, and V generated the most consistently identifiable peaks. Wave V was visible in all participants and in only three cases were Waves I or III not visible. Waves II and IV were absent more frequently with Wave II being absent in 40 cases and Wave IV absent in 42 cases. Waves II and IV were typically more difficult to find in participants over 45 years old. Waves I and V absolute peak latencies were prolonged by an average .1-.2msec per age group, and there was a statistically significant ($p < .01$) change between the 25–34 years old group and 55–75 years old group. As with the Konrad-Martin et al. (2012) study, Rosenhall et al. determined the Wave I-V interpeak latency did not shift with age. They also accounted for the hearing loss, which was often found in the older participants, by comparing results of individuals with hearing loss and those with normal hearing in the 25-34 and 55–75 years old group. They found no difference between the two groups, indicating the hearing loss did not impact wave latencies (Rosenhall et al., 1985). Changes in latency and amplitude could be seen in ABRs due to age alone. The studies done by Konrad-Martin et al. and Rosenhall et al. demonstrated latency changes across age groups. These changes were thought to occur in both the central auditory pathways and the peripheral auditory system. These studies indicated age could have impacts on objective test results; however, due to the ability to obtain behavioral thresholds in humans, the need to use toneburst ABRs in a threshold search has limited clinical use in adult humans. However, as behavioral testing is unreliable in canines, information about the auditory system and how it changes is usually obtained using objective testing.

Anatomy and Physiology of the Canine Auditory System

The canine ear is similar to the human ear; however, there are some clear differences. Canine pinnas differ from humans in shape as they can be either erect or pendulous. A pendulous pinna is folded over with the pinna covering the opening of the external auditory meatus and is seen in breeds such as beagles or spaniels. Breeds with pendulous pinnae are at higher risk for outer and middle ear infections. The erect pinna stands up with the opening of the ear canal unobstructed and is seen in breeds such as shepherds (Cole, 2010). Due to the complex array of muscles, most dog breeds can move their ears toward a sound source, which differs from humans who must move the entire head (Singh, 2017).

Like the human ear, the canine pinna is connected to the EAM. The border of the pinna leading into the ear canal is marked by three flaps of cartilage: the antihelix, tragus, and antitragus. The canine ear canal also creates cerumen but the canal typically has more hair that becomes denser the closer to the tympanic membrane it gets. Cerumen and the canal shape both serve to protect the ear just like in humans. However, the shape of the EAM is different in canines. While the human ear canal runs horizontally and is S-shaped, the canine ear canal has horizontal and vertical components and has an L-shape. Due to this unique shape with a 90° bend, visualization of the tympanic membrane during otoscopy can be difficult (Cole, 2010; Evans, 1993; Singh, 2017; Uemura, 2015).

The tympanic membrane (TM) in canines is like the TM in humans as it is a semitransparent membrane that separates the outer from the middle ear. Like the human TM, it has three membranous layers. The TM in canines has several of the same landmarks as the human TM such as the pars tensa and the pars flaccida. Some differences between the canine's TM and the human TM are there are less elastin fibers in the canine tympanic membrane than are

in the human tympanic membrane. In some dog breeds, the pars flaccida bulges even in the absence of a middle ear infection. The pars tensa of the TM connects with the manubrium of the malleus leading to the middle ear (Cole, 2010; Evans, 1993; Njaa et al., 2012; Singh, 2017; Uemura, 2015). Like humans, the canine middle ear starts at the tympanic membrane and houses three ossicles that are responsible for transmitting the sound from the tympanic membrane to the cochlea (Cole, 2010; Evans, 1993; Singh, 2017; Uemura, 2015). There are three spaces within the tympanic cavity: the epitympanic recess, the tympanic cavity proper, and the ventral cavity. The tympanic cavity proper is located next to the tympanic membrane. The malleus and incus are mostly contained within the epitympanic recess, which is the smallest area in the tympanic cavity. The ventral cavity is the largest of the three in the middle ear and connects with the tympanic cavity proper. Volume of the ear canal varies by breed and increases nonlinearly as body weight increases (Cole, 2010; Evans, 1993). The eustachian tube also connects the ear to the throat and equalizes pressure in the middle ear (Cole, 2010; Evans, 1993; Singh, 2017; Uemura, 2015).

The stapes footplate rests on the oval window and leads to the inner ear (Cole, 2010; Evans, 1993; Uemura, 2015). The canine inner ear also houses the cochlea and the vestibular organs. The cochlea is responsible for sending sound signals to the brain while the vestibular organs are responsible for balance just like in humans (Cole, 2010; Evans, 1993; Uemura, 2015). The canine cochlea is also a spiral shaped organ; however, it has 3¼ turns, which differs from the 2½ turns of the human cochlea. While the canine cochlea does have more turns, the structure and physiology of the cochlea is otherwise similar between canines and humans (Cole, 2010; Evans, 1993; Singh, 2017; Uemura, 2015). The cochlea houses the organ of Corti that contains the hair cells. The hair cells send the signal through the spiral ganglion and onto the

vestibulocochlear nerve (Uemura, 2015; Webb, 2009). Once the signal passes through the organ of Corti, it travels along a complex ascending pathway to the thalamus and the auditory cortex (Uemura, 2015). Several nuclei on the pathway help to preprocess sound before sending it up to the brain. The ascending pathway sends the signal along the auditory nerve to cochlear nuclei in the medulla oblongata. This location is tonotopically organized with the dorsal part of the nucleus representing the base of the cochlea and the ventral portion representing the apex of the cochlea. The cochlear nerve fibers terminate at the dorsal and ventral cochlear nuclei but fibers arise from the dorsal portion and form the acoustic stria. The acoustic stria lies on the dorsal side of the caudal cerebellar peduncle. The fibers of the acoustic stria split and become part of the lateral lemniscus. The fibers arising from the ventral cochlear nucleus move ventrally and form the trapezoid body. The dorsal nucleus of the trapezoid body receives excitatory input from both the contralateral and ipsilateral sides, which is crucial for localization. Some fibers continue past the trapezoid body as part of the lateral lemniscus while other fibers terminate in the dorsal nucleus of the trapezoid body. Fibers that travel with the lateral lemniscus reach the caudal colliculus, which helps integrate auditory information and is another crucial area for localization, and the medial geniculate nucleus of the thalamus, which processes and sends signals up to the primary auditory cortex. The primary auditory cortex is mainly located on the middle ectosylvian gyrus in the temporal lobe. The descending pathway follows a similar route and like the ascending pathway, not every fiber travels the same exact pathway. Most descending signals originate in the primary auditory cortex, the caudal colliculus, or the medial geniculate nucleus of the thalamus. A signal originating in the primary auditory cortex would then be sent to the caudal colliculus. From there, the signal is either sent directly to the cochlear nuclei or it

indirectly passes through the dorsal nucleus of the trapezoid body. The efferent fibers travel ipsilaterally to the IHCs (Uemura, 2015).

For canines, the afferent pathway can also be assessed using AEP testing just as it can for humans. When performing ABR testing on animals, the term brainstem auditory evoked response (BAER) testing is more commonly used. The ABR and BAER tests are essentially the same but the BAER was used for the remainder of this paper when referring to testing in canines.

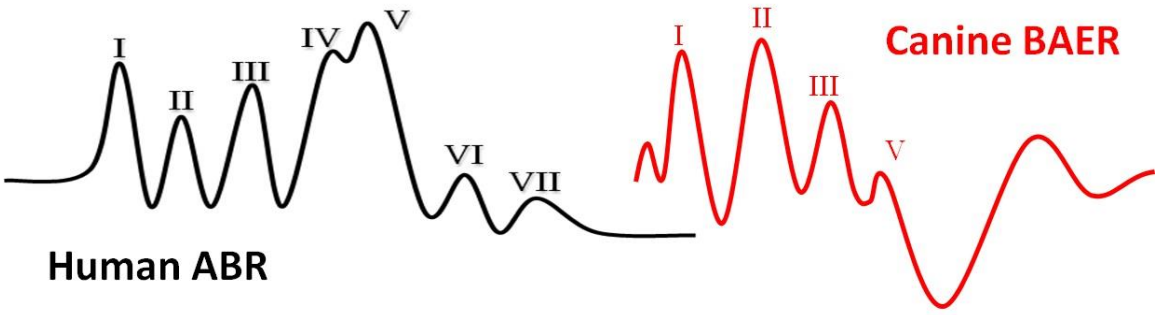
Brainstem Auditory Evoked Response Testing

Assessments of the canine auditory system are limited. Behavioral testing in canines is unreliable and is therefore not a means for assessing canine hearing. However, BAER testing can be performed with canines without the use of anesthesia. Brainstem auditory-evoked response testing is an objective, electrophysiologic test that can assess the integrity of the auditory system as well as estimate the hearing thresholds of the animal being used to test (Scheifele & Clark, 2012).

Canine BAER morphology differs somewhat from human ABR morphology. For example, results do not always have five waves present but Wave V is often visible and typically the wave used for measuring thresholds (see Figure 3).

Figure 3

Schematic of Human Auditory Brainstem Response Waveform Versus Canine Brainstem Auditory-Evoked Response Waveform



Note. Figure drawn by Tina Stoody.

While stimulus intensity has the same impact on canine BAER waveforms as it does the human ABR, animal testing involves different units of measurement (Scheifele & Clark, 2012). Stimulus intensity for ABR testing is measured using dB nHL; however, this is not a way to measure intensity in animals because it is normed for humans. For canine BAER testing, peak sound pressure (dB peSPL) is used instead. Peak sound pressure is generated from the click stimulus. "The peSPL is the maximum absolute value of the instantaneous sound pressure in the click interval" (Scheifele & Clark, 2012, p. 1246). The dBnHL to dBpeSPL conversions vary slightly between different pieces of equipment but generally there is about a 30 dB difference, such that the recommended levels for diagnostic testing (70-116 dBpeSPL) are equivalent to about 40 to 86 dB nHL (Scheifele & Clark, 2012).

Head size might impact latencies according to Meij et al. (1992); however, two additional research groups found head size had no impact on BAER results (Kemper et al., 2013; Munro et al., 1997). Unlike humans, there was no evidence sex impacted canine BAER test results. State of arousal and sedation also had no impact on test results (Scheifele & Clark, 2012; Wilson &

Mills, 2005). Artifact from muscle movement near the head or jaw can impact BAER results by leading to excessive noise which may invalidate results. If the amount of artifact is problematic, then use of physical restraint may be necessary (Hall, 1992; Wilson & Mills, 2005).

Human ABR testing typically involves the use of surface electrodes that adhere to the skin; however, this is not possible with canines due to substantial fur. Therefore, subdermal needle electrodes are typically used for canine BAER testing. The non-inverting electrode is placed at the vertex of the canine's head. The inverting electrode is placed inferiorly and anteriorly to the ear being stimulated and the ground electrode is placed inferiorly and anteriorly to the contralateral ear (Scheifele & Clark, 2012; Wilson & Mills, 2005). Like humans, insert transducers can be used. The same filter settings used in human testing could be used with canines with a recommended high pass filter of 150Hz and a low pass filter at 1500Hz (Scheifele & Clark, 2012). Shorter latencies and clearer morphology have been found in canines when using condensation polarity; however, the results were not significant (Kawasaki & Inada, 1994).

Stimulation rate is important in canines. A faster stimulation rate leads to quicker test times. Quicker test time reduces the amount of stress the dog undergoes being tested. However, just like in humans, a faster stimulation rate could lead to poorer morphology. To address this, Scheifele and Clark (2012) recommended using a stimulation rate of 33.3 clicks per second to maximize time efficiency and waveform morphology.

Tone bursts and clicks are both appropriate stimuli to use when assessing canine hearing. In the study by Ter Haar et al. (2002), tonebursts from 1-32kHz and click stimuli were used during BAER testing. The researchers in this study examined thresholds, latencies, and amplitudes in 10 dogs between the ages of 3.5 and 7 years old using both click and toneburst stimuli from 1k-32kHz. Testing was done on sedated canines; each stimulus was tested first at

80dB SPL to obtain clear waveforms in order to document each peak. A threshold search was performed by decreasing intensity by 10dB until there was no repeatable Wave V; threshold was marked 5 dB above the no response. The researchers reported that the best thresholds in this age group were found using clicks, 12kHz tonebursts, or 16kHz tonebursts compared to thresholds from other toneburst frequencies tested. The average click threshold was .5dB SPL with a standard deviation of +/-14.5. The mean threshold for 12kHz was -3.5dB SPL with a standard deviation of +/-16.3 while the mean 16kHz threshold was 5.5dB SPL with a standard deviation of +/-20.6. The findings of this study suggested the most sensitive hearing thresholds were best detected when using one of those three stimuli in middle age canines (Ter Haar et al., 2002). Clicks provide a robust response and could assess more of the auditory system but when looking for frequency-specific information, tonebursts are preferable. Toneburst results could have different latencies and thresholds as well as smaller amplitudes compared to clicks but they could provide more frequency-specific information.

Except for the protocol recommendations cited above from Scheifele and Clark (2012), currently there is not a universally agreed upon protocol for testing canines in the literature, and there is a high amount of variability across published studies in terms of protocol. This lack of standardized protocols makes interpreting results more difficult when there is no standard with which to compare results (Scheifele & Clark, 2012).

Applications for Brainstem Auditory-Evoked Response Testing

Assessment of puppy hearing to rule out congenital hearing loss is the most common application of BAER testing currently. Puppy hearing screenings are mostly used for breeds at higher risk for congenital hearing loss such as dalmatians. Puppy screenings are typically done at a high intensity like a neurodiagnostic ABR. In puppy screenings, a click stimulus at high

intensity is often used because a hearing loss across the frequency range is more typical for a congenital loss. Brainstem auditory-evoked response testing can also be used to assess adult and elderly canine hearing. Clicks can be used in BAER testing; however, the use of toneburst stimuli is also of interest as it might be able to give more frequency-specific information. It is likely certain frequencies might be more impacted by aging or noise such that click evoked BAER responses might not be as sensitive to smaller changes in hearing sensitivity than tonebursts. Testing in older dogs is not limited to breeds at risk but could be used to help family pets or working dogs such as police, military, or service dogs.

The Orthopedic Foundation for Animals (OFA, 2020) has published requirements to certify BAER tests but these requirements are not sufficient in several situations. The OFA requirements are most appropriate for younger dogs and do not address threshold searches or best practice for older dogs. The requirements indicated an animal should be 35 days or older before undergoing a hearing screening. Insert earphones were recommended. Signal intensity was recommended between 70 and 105 dB nHL. The OFA recommended averaging at least 200 clicks when obtaining a response. These parameters are slightly problematic as using dB nHL is not appropriate for animals as it is normed to humans. A more appropriate recommendation should use dB peSPL, which is the appropriate unit to use with animals. In addition, 200 clicks are not likely to produce waveforms with clear morphology. The minimum number of clicks for an average should be closer to 700 or 800 clicks. As stated above, these recommendations are also only appropriate for a screening protocol; they would not be optimal for diagnostic testing to estimate hearing thresholds in canines.

Presbycusis in Canines

The canine auditory system experiences physiologic changes with age comparable to the physiologic changes age inflicts on the human auditory system. While research into effects of presbycusis on the canine ear and BAER results are limited at this time, a study by Shimada et al. (1997) was performed to determine the impacts aging had on the canine ear. Twenty-three dogs were involved in the study; their ages ranged from three days to 17 years old. Each dog in the study had their hearing assessed by watching for behavioral changes in response to claps as well as BAER testing. The BAER testing was done at 90 dB using a click stimulus. No note was made if the testing was done using dB SPL, nHL, or peSPL. While the results still showed changes to the auditory system due to age, it was difficult to compare hearing thresholds to other studies without knowing which dB was used. Changes in the human cochlea, loss in spiral ganglion, atrophy of the organ of Corti and stria vascularis, and thickening of the basilar membrane were looked for in the canines. A histological examination of the canine auditory system revealed these same physiologic characteristics occurred in dogs, especially those over 12 years of age. Some dogs even showed a complete destruction of the organ of Corti and changes to the cochlear nuclei including nerve cell loss. The aging process of the ear appeared to begin around 10 years old. The authors also found damage to the cochlea was often accompanied by hearing loss; however, the amount of cochlear damage did not correspond to the severity of the hearing loss. The authors did not define the level of hearing loss found or how it correlated to the damage found in the cochlea. They did state that canines did appear to have different types of presbycusis like humans (Shimada et al., 1997).

Maturation of the Brainstem Auditory-Evoked Response

Like humans, evoked responses take some time to develop into adult-like responses. Poncelet et al. (2002) performed a study to see how the BAER developed in puppies at specific frequencies. They used nine beagle puppies from two different litters and split them into two groups. The first group was tested at 10, 13, 19, 25, and 45 days post birth while the second group was tested on the 16th day. They then took two puppies from Group 2 and two additional puppies and tested them between days 42 and 47 to get data for slightly older puppies. This additional group's data were added to the Group 1 puppies' day 45 data. Testing was done while the puppies were sedated. The researchers placed the noninverting electrode at the vertex of the skull, the inverting electrode on the ipsilateral mastoid, and the ground on the neck. Their filter was set from 15 to 1,500Hz. Threshold search BAER tests were performed for .5k, 1k, 2k, 4k, 8k, 16k, and 32k Hz. There were 1,000 sweeps during each intensity level; however, they only used 250-500 sweeps for the highest frequencies. Rarefaction polarity was used with a 9.6Hz repetition rate. When recording, they began at the maximum intensity and decreased intensity by 10dB for each repeatable response. Threshold was defined as halfway between the last repeatable response and no response. They tested frequency in random order to prevent any physiology impacts on the data.

Poncelet et al. (2002) found BAER responses were detectable around the 13th day with responses stabilizing around 19-25 days. The 1.5-year-old group's thresholds were best in the 2k - 8k Hz range. Thresholds worsened by about 11dB each octave below 2k and worsened by about 20dB for each octave above 8kHz. These results were different from the Ter Haar et al. (2002) study in which the best thresholds were found using a click and 12kHz and 16kHz toneburst stimuli. However, the dogs in the Ter Haar et al. study were ages 3.5 to 7 years while

the oldest dogs in the Poncelet et al. study were only 1.5 years old. Maturation and age might have played a role in the difference between these two studies.

Poncelet et al. (2002) found the development of the audible frequency range followed a similar pattern to a previous study done with kittens. The most sensitive frequency was 4kHz on day 10 but by day 15, the kittens and puppies had reached their maximum sensitivity to thresholds below 1kHz. Day 20 saw maximum sensitivity up to 20kHz and by one month, both kittens and puppies were adult-like in the audible frequency range (Ehret & Romand, 1981; Poncelet et al., 2002). The authors stated that their results corresponded with the maturation of click thresholds in other studies (Poncelet et al., 2002).

Presbycusis and Brainstem Auditory-Evoked Response Testing

Presbycusis in canines is beginning to become more recognized by experts as an acquired sensorineural hearing loss. Much like in humans, it is typically a symmetrical loss (Strain, 1996; Ter Haar et al., 2008, 2009). Ter Haar et al. (2009) found changes occurred in the cochlea as canines aged. The researchers evaluated 10 geriatric dogs with a mean age of 12.7 years and a control group of three puppies who were nine months old with normal hearing. Brainstem auditory-evoked response testing was performed using toneburst stimuli at each octave ranging from 1kHz to 32kHz. Testing started at an intensity level of 80 dB SPL for each frequency and was reduced in 10dB SPL steps until the threshold was reached. The researchers defined the threshold as 5 dB above the lowest intensity level with no identifiable Wave V. If no response was found at 80 dB, then the intensity was increased by 10dB until threshold was found or 100 dB was reached. After the testing was completed, the animals were humanely euthanized to further study the cochlea.

Results of the histological examination showed cochlear lesions in the geriatric group. There was damage to the outer and inner hair cells as well as the spiral ganglion cells. The hair cell damage varied by dog; however, Ter Haar et al. (2009) stated that rows 1 and 2 of the OHCs were more often damaged than row 3. Inner hair cell loss occurred as well but loss was more severe in the OHCs. Hair cell loss was also found to be more severe in the basal turn than the apical turn. There was also damage to the strial cells and an overall reduction in the stria vascularis. The authors indicated the damage stated above was common in all the aging dogs. The findings from the postmortem histological examination corresponded well with antemortem BAER test results (Ter Haar et al., 2009). They found the geriatric group had worse BAER thresholds across the frequency range when compared to the thresholds of the puppies and the greatest changes to thresholds occurred between 8kHz and 32kHz. While Ter Haar et al. stated 12kHz and 16kHz were the most impacted by age, they did not clarify on how much of a difference existed.

Ter Haar et al. (2008) performed a longitudinal and cross-sectional study on canines to examine how aging impacted the canine ear and BAER results. The cross-sectional study examined how hearing thresholds varied among the three groups while the longitudinal study examined how thresholds changed in Group II as the dogs aged. In the cross-sectional portion of this study, they used three groups: young, middle aged, and elderly. The young group (Group I) had a mean age of 1.8 years; the middle age group (Group II) had a mean age of 5.7 years and was the group used for the longitudinal study. The last group (Group III) was the geriatric dogs whose mean age was 12.7 years. Since different sized dogs have different lifespans, dogs of similar sizes were used. Thresholds were obtained during sedated BAER testing using click and toneburst stimuli at 1, 2, 4, 8, 12, 16, 24, and 32 kHz. Thresholds were obtained by starting at

80dB SPL and decreasing by 10dB with each repeatable response until there was no recognizable Wave V. If no threshold was obtained at 80 dB, then the intensity was increased by 10 dB until threshold was found or 100dB was reached. If no response was found at 100dB, then the researchers decided to assign 100dB as threshold. Thresholds were found in all dogs except for in one geriatric dog (age: 13.9 years) at 32kHz. The thresholds between Groups I (young) and II (middle age) were similar while Group III (geriatric) had significantly higher threshold levels than Groups I and II. Groups I and II had average thresholds ranging between 0dB SPL and 50dB SPL with the best thresholds between 8 and 16kHZ. Group III had a flatter configuration with the average thresholds ranging between 60dB and 80dB SPL. The changes in threshold were most pronounced between 8 and 32kHz, with 12kHz and 16kHz the most impacted, while thresholds from 1-4kHz had less pronounced differences. No differences were found between ears (Ter Haar et al., 2008). This study was unique because BAER research on dogs typically does not include higher frequency stimuli and this study has not been replicated yet. A summary of the results found by Ter Haar et al. (2008) can be found in Table 1.

Table 1

Summary of Ter Haar et al. (2008) Findings

Group 1 (Young Dogs)	Group 2 (Middle-Aged Dogs)	Group 3 (Geriatric Dogs)
• Thresholds ranged from 0-40dB across frequencies • Reverse cookie bite configuration with best thresholds from 4kHz to 16kHz	• Similar thresholds levels (0-50dB) to group 1 except at 4k • Similar shape to hearing configuration as group 1with best thresholds from 8kHz to 24kHz	• Thresholds ranged from 60dB to 80dB • Flatter configuration than group 1 and 2 • Biggest difference from group 1 and 2 was seen when using click, 12kHz, and 16kHz

In the longitudinal study (Ter Haar et al., 2008), the dogs from Group II of the cross-sectional study continued to have their hearing tested once every one to two years for seven years to track changes within the individual dogs' auditory systems. Thresholds were obtained using the same testing methods used in the cross-sectional study. The researchers found three of the dogs in Group II had no change to their thresholds; however, eight of the dogs in the group had poorer thresholds at the end of the study. This change in threshold was progressive and occurred most commonly between 8 and 10 years old. The researchers also noted the rate of hearing loss changed and severity of thresholds differed dog to dog. These results indicated the dogs experienced progressive higher frequency hearing loss characteristic of presbycusis (Ter Haar et al., 2008).

Presbycusis does not just impact the ability to hear. In humans, it can impact mental health and social activities, and it is thought to have an impact on the behavior of canines as well (Houpt & Beaver, 1981; Scheifele & Clark, 2012; Strain, 1996). They may become more depressed and lethargic as their hearing and auditory pathways decline. Safety can also be a concern; dogs with hearing loss are at higher risk for getting lost or injured. It is also important for working dogs to be able to hear not just for their safety but their handler's as well (Houpt & Beaver, 1981; Scheifele & Clark, 2012; Strain, 1996).

In conclusion, the auditory system is a complex system that is impacted by the aging process. While behavioral testing is inappropriate to use with canines, electrophysiologic tests can assess canine auditory systems. In humans and canines, presbycusis is typically found first in higher frequencies above the 2-4kHz range. Due to the larger range of hearing dogs have, it stands to reason that hearing needs to be evaluated at higher frequencies than is typically done in standard audiometric testing. Further research needs to be performed to determine which

frequencies are most impacted to create an appropriate protocol for diagnostic testing of age-related hearing loss in canines. The aim of this study was to determine if a high frequency toneburst, rather than the more commonly used click stimulus, might be better able to differentiate hearing loss in older canines via differences in brainstem auditory evoked responses (BAER) due to the pattern of hearing loss beginning in higher frequencies.

CHAPTER III

METHODOLOGY

Subjects

Ten healthy dogs aged nine years or older underwent BAER testing in this study. Convenience sampling methods were employed, and recruitment efforts were carried out via word of mouth, fliers, social media, and with the assistance of several local veterinary offices. Exclusionary criteria were positive history of noise exposure, presence of otic or neurological disease, known congenital hearing loss, or use of ototoxic medication. Consideration was also given to weight of the dog; dogs under 20 pounds and over 100 pounds were excluded due to the influence size has on life expectancies, which would impact the age at which they are determined to be geriatric (Ter Haar et al., 2008). Dog breed and gender were not exclusionary criteria as it has been determined that breed and gender do not impact BAER results (Kemper et al., 2013). A detailed case history was taken with each dog's owner prior to scheduling testing to rule out exclusionary conditions and verify each dog met qualification criteria for the study. Breeds that participated in this study included Labradors, German Shepherds, Huskies, and mixed breeds. Performance of BAER testing on canines at the Facility for Education and Testing of Canine Hearing and Laboratory for Animal Bioacoustics at the University of Northern Colorado (FETCHLAB UNC) was approved by the Institutional Animal Care and Use Committee, which was responsible for determining research was ethically and humanely conducted (see Appendix A).

G*power was used to calculate an appropriate sample size. The significance level (α) chosen was .05 with a desired power of .95. The minimum sample size needed was calculated to need 8-10 dogs. Ten dogs were tested in both conditions with two being excluded from the study due to having no responses at the highest intensity using a click.

Preparation for Testing

Prior to testing, a wellness check was performed on each animal by the principal investigator. If any concerns arose in the wellness examination that would have impacted testing or put the health of the animal at risk, the dog would be dismissed from the study and the owner given an explanation. Every participant was healthy enough to undergo testing. To make the participant more comfortable during electrode insertion, lidocaine/prilocaine cream, a topical numbing agent (2.5% lidocaine, 2.5% prilocaine), was placed at the vertex of the dog's head between the ears and rostral to each tragus where the electrodes were placed. Owners of each participant signed a consent form prior to testing that explained the process of testing and provided information about participation (see Appendix B). A pre-determined checklist was created by the primary investigator and was followed for each testing session so correct and consistent methods were ensured each time a dog was tested (see Appendix C).

Recording Procedure

The Intelligent Hearing Systems USB Box or stimulus generator with Smart EP software was used during data collection. Calibration using American National Standards Institute (2018) Standards S3.6 occurred less than one year prior to data collection. A Thundershirt, a wrap that applies a calming pressure to the animal, was used to help keep the animal more relaxed and can be seen in Figures 4 and 5. Disposable 13 mm subdermal bent needle electrodes with a 0.4-

millimeter diameter were used to record electrical changes (see Figure 4 for participants being prepped and tested).

Figure 4

Two Test Participants

Note. Participant being prepped on the left. Arrows point to electrode locations. Participant being tested on the right.

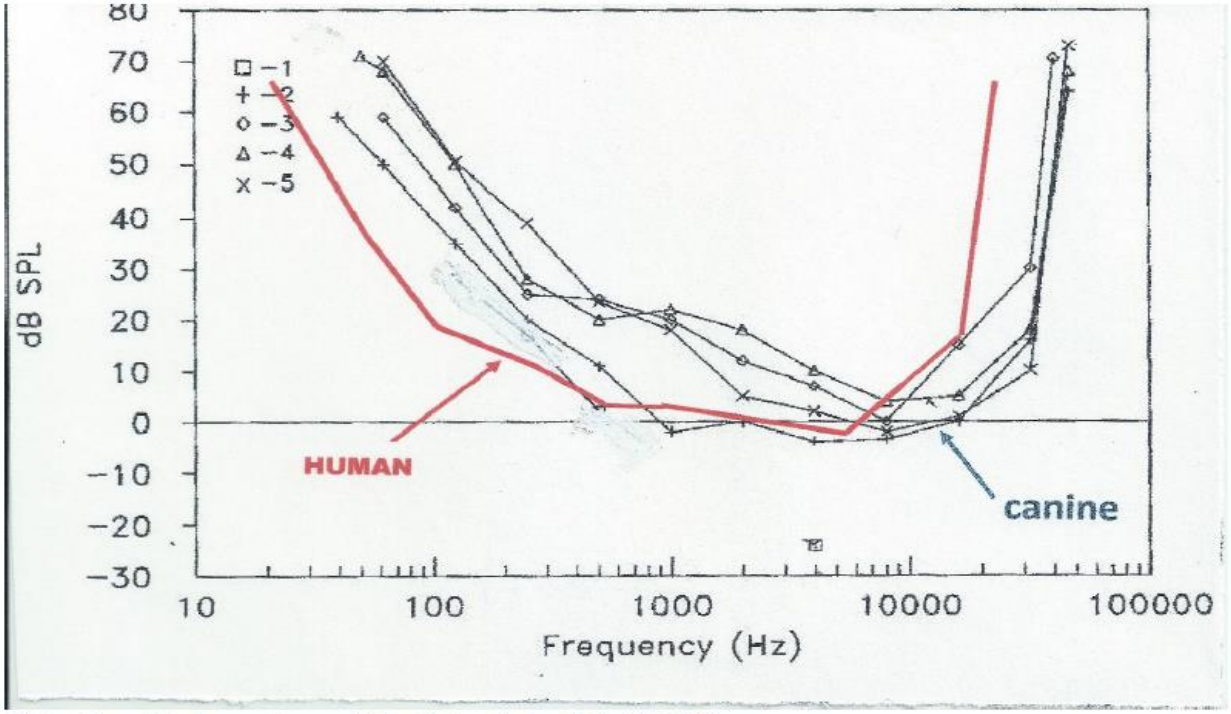
The three locations for electrode placement were as follows: vertex of the scalp (Cz), rostral to the base of the test ear (Ai), and rostral to the base of the non-test ear (Ac). The vertex placement was the non-inverting, positive electrode. The test ear was the inverting, negative electrode and the opposite ear was the ground/common electrode. Electrode impedances were measured at 3k ohms or less. The stimuli used were clicks and 12kHz tonebursts as Ter Haar et al. (2002, 2008) demonstrated the biggest changes over time could be seen using click and 12 or

16kHz toneburst stimuli. Better thresholds were found at 12kHz over 16kHz in younger dogs (Ter Haar et al., 2002) and larger changes in threshold for older canines were seen when using 12kHz rather than when using 16kHz (Ter Haar et al., 2008) so a 12kHz toneburst was chosen as the stimulus to compare with a click. A behavioral minimal audibility curve showed similar thresholds could be seen in younger dogs at 12kHz and in the frequency range that clicks best assessed (see Figure 5). While there was some disagreement on the exact thresholds between Ter Haar and the minimal audibility curve, the data suggested similar thresholds in younger canines when using 12kHz and clicks. In addition, thresholds were collected at the FETCHLAB UNC facility prior to the current study from a seven-week-old puppy using a click and tonebursts including 12kHz. Threshold levels were similar using both 12kHz and clicks in the young dog and were comparable to those on the behavioral minimal audibility curve. Similar thresholds levels between the two stimuli in the current study could allow comparison between click and 12kHz thresholds. Thresholds from seven-week-old canine can be seen in Figure 6.

The clicks used in the current study had a duration of 100usec with a rectangular envelope, and the 12kHz tonebursts had a duration of 5000usec with a Blackman envelope (see Figure 7 for the spectral information).

Figure 5

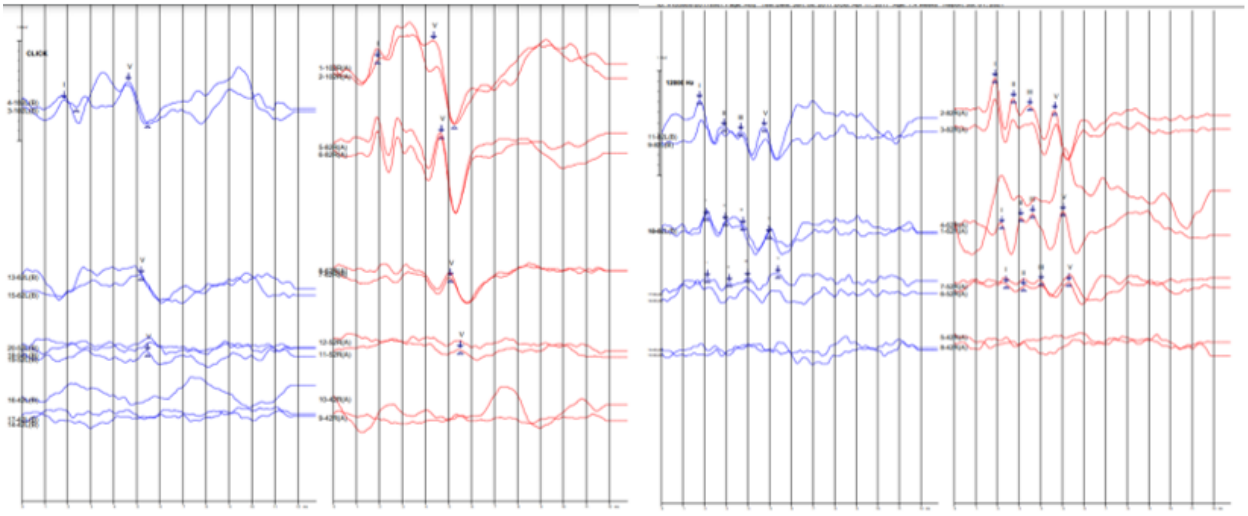
Human and Canine Minimum Audibility Curve



Note. Provided courtesy of Dr. Peter Scheifele (2020).

Figure 6

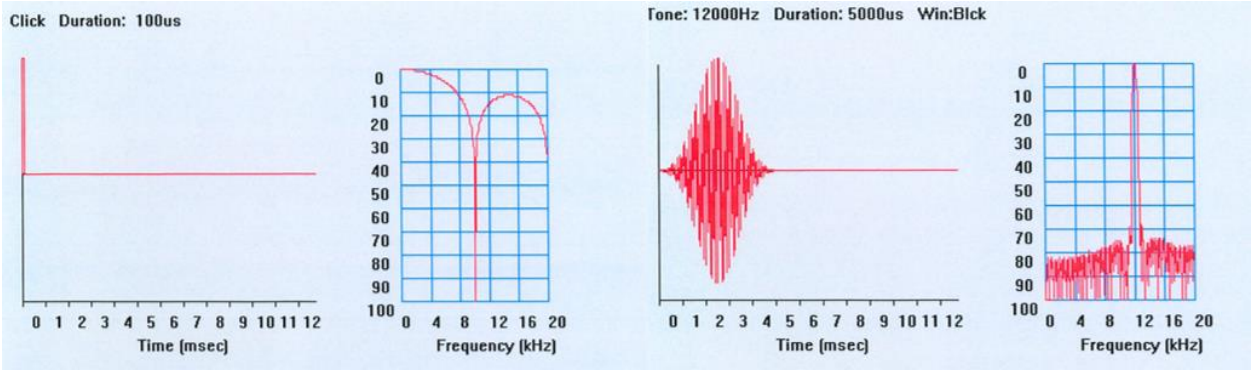
Thresholds Using Clicks and 12kHz Tonebursts



Note. Click thresholds (left ear and right ear) on the left; 12kHz toneburst thresholds (left and right) on the right.

Figure 7

Waveform and Spectrum for Each Stimulus



Note. Click waveform and spectrum on left, 12kHz toneburst waveform and spectrum on right.

The stimuli were presented to the test ear using an Etymotic ER-2 transducer and foam insert earphones to couple to the dog's ear. The stimuli were determined based on findings in multiple Ter Haar et al. (2002, 2008, 2009) research studies which indicated that in young dogs, thresholds were best when using clicks, 12kHz, and 16kHz, and in older dogs the thresholds using these three stimuli showed the most change over time. The purpose of the current study was to look at how click thresholds compared to a high frequency toneburst threshold. As reported by Ter Haar et al. (2008), thresholds using 12kHz showed more changes over time than the 16kHz toneburst so a 12kHz toneburst was chosen in the current study as the stimulus to compare thresholds with the click. The BAER testing was conducted on the more accessible ear of each participant as Ter Haar et al. (2008) demonstrated there was no significant difference between ears as aging occurred. The time window was 10ms. The low pass filter was set at 1.5k Hz and the high pass filter was set at 150 Hz. The artifact reject window was 35%. The stimulation rate was 33.3 clicks per second (Scheifele & Clark, 2012). An attempt was successfully made to collect 1,000 sweeps for each dog but in five cases, testing was terminated prior to 1,000 sweeps due to generation of excess artifact from subject restlessness. No waveform had less than 600 sweeps. After the initial runs were performed, the waveforms were averaged for clearer waveforms to better track Wave V. In addition, latency and amplitude were determined from the averaged waveform. Each averaged waveform had at least 1600 sweeps.

An initial click stimulus was presented at 92 dB peSPL to verify adequate auditory function of the ear. If no response was obtained from the 92 dB peSPL presentation, then the stimulus was presented at 102 dB peSPL. In the event of no-response, 112 dB peSPL was marked as threshold for calculation purposes. If responses were obtained, the threshold search was performed. Waveforms were determined based on latency, clear morphology, and

repeatability. During the threshold searches, small changes in latency and morphology as intensity decreases should be present. A clear peak between four and fivee milliseconds at a high intensity was marked as a response. When waveform morphology was repeatable and peaks were easily identifiable, results were accepted after one or two runs. Closer to threshold, when morphology was less distinct, two or more runs were conducted to ascertain a valid and repeatable peak had been identified. If still unclear, aspects of the waveform such as latency, amplitude, and artifact were also considered when determining if Wave V existed and was repeatable. Latency was reviewed to ensure the waveforms were marked correctly and the timing made sense for Wave V. Morphology was less used; however, waves with small amplitudes were discussed and even led to certain waveforms being considered no responses. Waves were marked at the shoulder of each peak to the trough of the peak. Agreement was reached between the principal investigator and at least one committee member on potential Wave Vs before decreasing intensity. Intensity was reduced by 20 dB until no response was obtained. Once no response was present, the intensity was increased by 10 dB. The threshold was marked as the last intensity level with a repeatable response.

The following is an example of the test procedure:

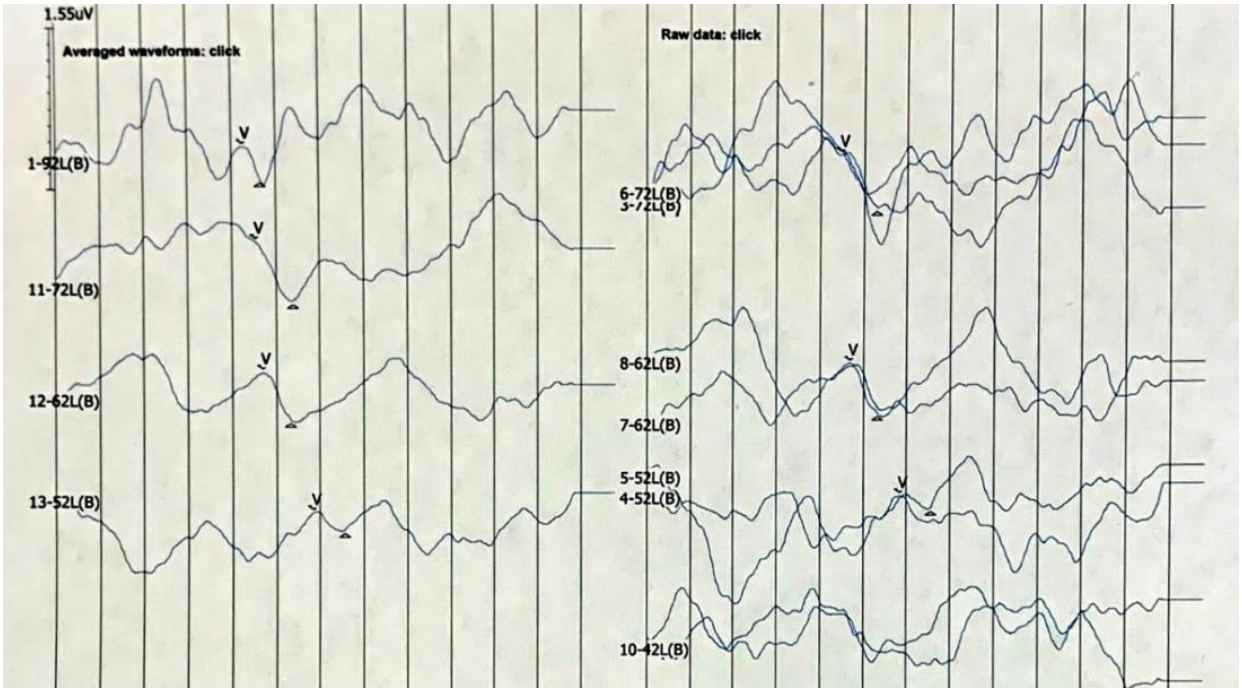
92dB SPL (response) → 72dB SPL (response) → 52dB SPL (response) → 32 dB SPL (no response) → 42 dB SPL (response= threshold)

Once the click threshold was obtained, the tester switched to the high frequency toneburst stimuli. 12k Hz tonebursts were presented at 92dB SPL. The toneburst threshold searches followed the same procedure to find thresholds as the procedure used to find threshold in response to clicks. After the data were collected, the waveforms were averaged; the averaged waveforms were used to determine amplitude and latency and were also used to see the Wave V

changes as threshold decreased in an easier manner. Representative waveforms from this protocol can be seen in Figures 8 and 9. Waveforms were reviewed multiple times after the conclusion of testing to ensure the thresholds were marked correctly, latencies and amplitudes were appropriate for the intensity level (i.e., lower thresholds should have longer latencies), and there were no errors during testing that would nullify responses.

Figure 8

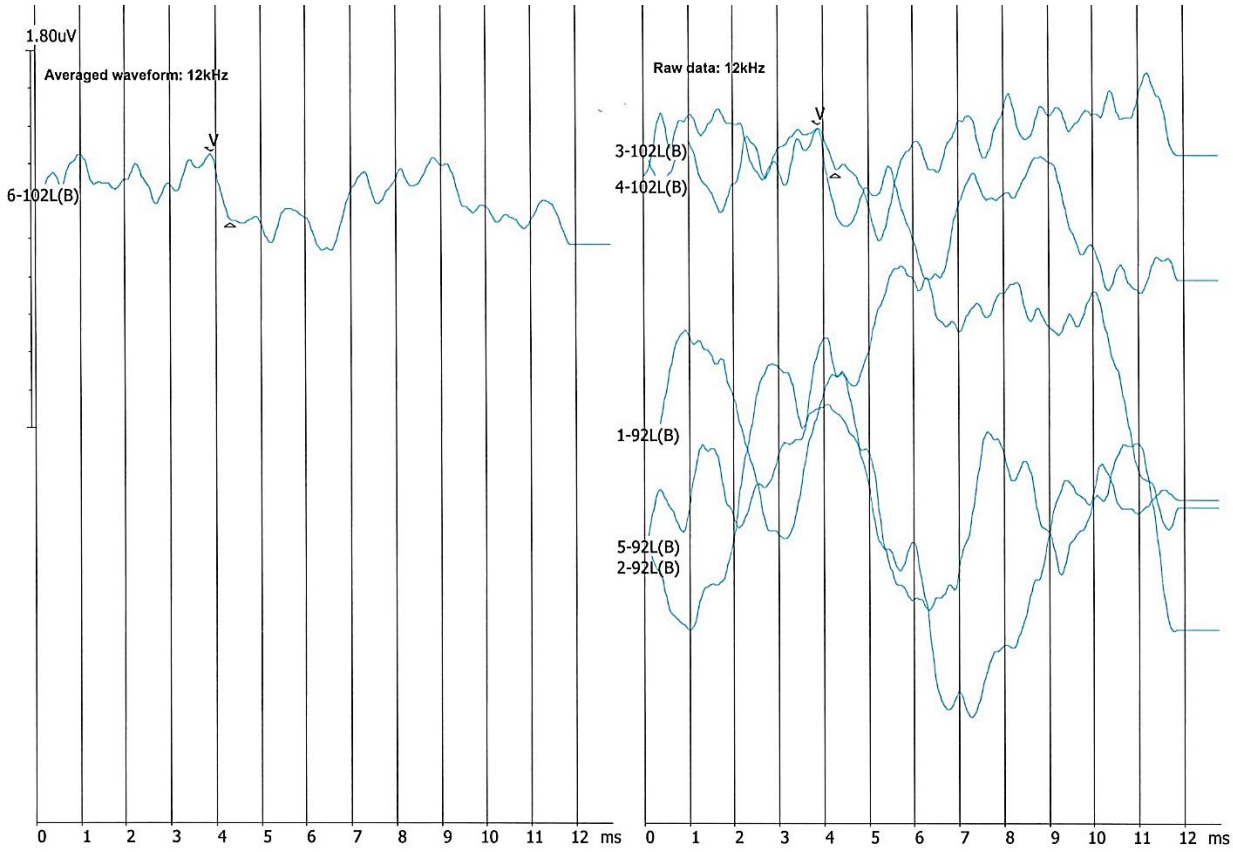
Averaged Waveforms and Raw Data Using a Click Stimulus



Note. Click averaged data on left. Click raw data threshold search on right.

Figure 9

Averaged Waveforms and Raw Data Using a 12kHz Toneburst



Note. 12kHz averaged data on left. 12kHz raw data threshold search on right.

CHAPTER IV

RESULTS

The purpose of this study was to determine if there was a difference in the thresholds for a click and a high frequency toneburst. Ten healthy canines of various breeds were recruited and participated in the study. The participants' ages ranged from 9 to 13 years. Dogs under nine years of age were not considered for the study. The mean age was 10.8 years with a standard deviation of +/- 1.619 and a range of 9-13 years. Participant thresholds using a click and 12kHz toneburst were identified in one session that lasted 45 minutes or less. Table 2 provides demographic information for the participants.

Table 2

Participant Demographics

Participant	Age (Years)	Breed	Sex
1	12	Labrador	Female
2	9	Labrador	Male
3	13	Labrador	Male
4	9	Mix	Male
5	12	Huskey	Male
6	11	Huskey	Female
7*	12	Labrador	Female
8*	12	German Shepherd	Female
9	9	German Shepherd	Male
10	9	Mix	Female

Note. * indicates dogs removed from study.

Statistical Analysis

As previous studies by Ter Haar et al. (2002, 2008, 2009) demonstrated, the biggest changes in thresholds due to age were found by using a 12kHz toneburst stimulus and a click

stimulus in canines. In this study, a paired *t*-test was performed to determine if there was a significant difference between average threshold levels of the two stimuli (click and 12k) using BAER testing. A paired *t*-test was chosen to compare the two mean thresholds of the same test. The 12kHz toneburst was thought to show the typical high frequency loss associated with the aging process better than the click. The stimulus type was the independent factor and the Wave V threshold for each stimulus type was the dependent factor. Wave V thresholds for a 12kHz toneburst and a click were compared. Latencies and amplitudes were documented and a descriptive analysis of these measurements was completed.

Summary of Results

Thresholds were compared using two different stimuli: a click and a 12kHz toneburst. Ter Haar et al. (2002) found waveforms with similar thresholds when using a click, 12kHz, or 16kHz in young dogs, and a separate behavioral minimal audibility curve demonstrated similar thresholds between 12kHz pure tones and thresholds in the 2-4kHz range for young canines (Scheifele, 2020). Ter Haar et al. (2008) found in canines that thresholds using these same stimuli were most impacted by age. The current study attempted to see if there was a significant difference in thresholds between a click and a high frequency toneburst; results showed statistically significant higher thresholds were found using a 12kHz rather than a click in dogs nine years and older. Latency and amplitude were documented.

Descriptive Analysis

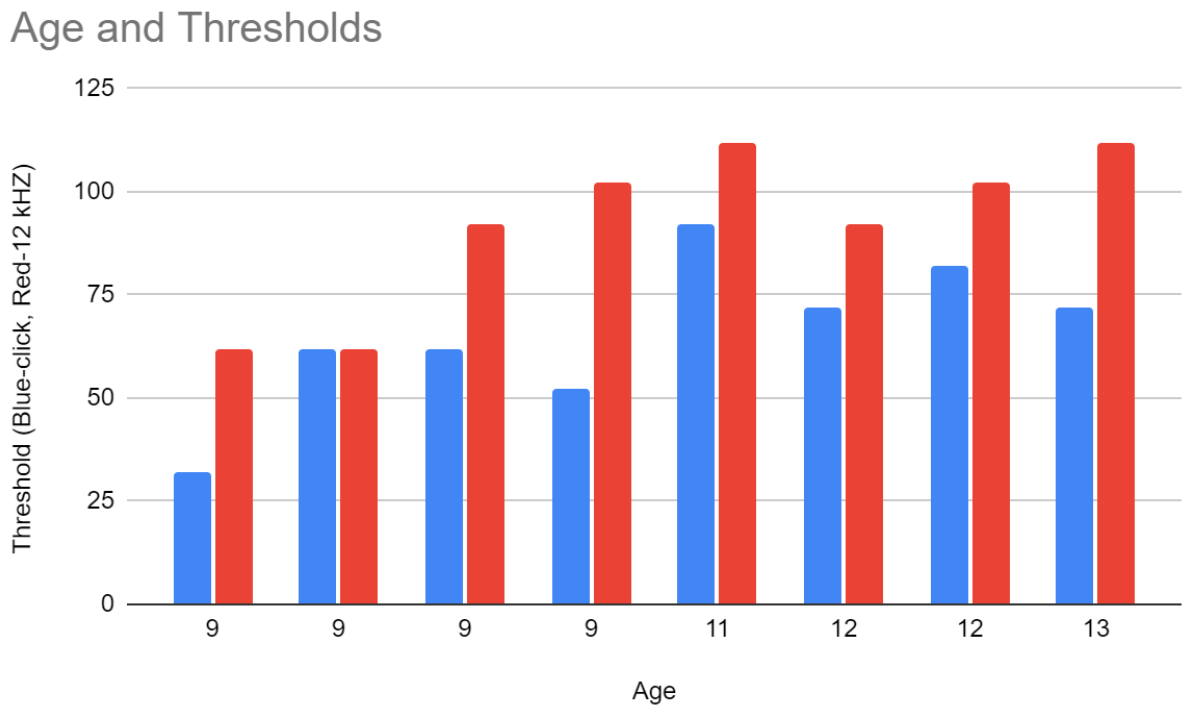
Wave V Thresholds

Hearing thresholds were obtained using a click stimulus and a 12 kHz stimulus in 10 aging dogs. Wave V was first identified at a high intensity and then was tracked as intensity decreased until Wave V was no longer identifiable. After testing, two dogs were removed from

the data analysis because threshold levels could not be agreed upon by researchers and concerns of either a no response, test artifact, or conductive component could not be ruled out. Statistical analysis was reported for the eight dogs and results of the study showed a significant difference between a click and 12kHz thresholds. There was an effect size of .997 with the data analysis of the eight dogs. The average click and 12 kHz threshold were compared and thresholds for each dog were organized by age (see Figure 10).

Figure 10

Thresholds for Click and 12 kHz (in dB peSPL) Organized from Youngest Participants to Oldest



Click

Using a click, Wave V was identified at 92 dB peSPL in all but two dogs. The two dogs without a response were removed from the study for the reasons stated above. The average threshold using a click was 65.75 dB with a standard deviation of +/- 18.5. The threshold range was 60 dB with the worst thresholds at 92 dB and the best thresholds at 32 dB. Appendix D provides the averaged click threshold searches for each participant.

12,000Hz Toneburst

The average threshold using a 12kHz toneburst was 92.00 dB peSPL with a standard deviation of +/- 20. The threshold range was 50 dB with thresholds ranging from 62 to 112Db SPL. Thresholds for both click and 12kHz are recorded in Table 3. While the primary consideration was threshold, latency and amplitude were also documented to show the parameters that helped to determine Wave V. Appendix E shows the averaged 12kHz tonebursts for each participant.

Table 3

Thresholds for Click and 12kHz Toneburst Stimuli (in dB peSPL) for Ten Dogs

Test Subject/Ear Tested	Click Threshold	12kHz Threshold
1/Right	72dB	92dB
2/Right	62dB	62dB
3/Right	72dB	112dB
4/Right	32dB	62dB
5/Right	82dB	102dB
6/Right	92dB	112dB
9/Right	62dB	92dB
10/Left	52dB	102dB

Note. Dogs 7 and 8 were excluded from data analysis.

Latency

Click

Latency was compared for a click at 92dB peSPL. The average latency at 92dB peSPL was 4.58ms. As thresholds varied by dog, the average latency at threshold was unable to be compared. The range for latency at 92dB was .65ms, ranging from 4.38ms to 5.03ms. The standard deviation for latency at 92dB was +/-.20. The latency for each participant using a click at 92dB is documented in Table 4.

12kHz Toneburst

Latency was also compared for the 12kHz toneburst at the high intensity. The average latency at 92dB peSPL was 4.70ms. The range for latency at 92dB was 1.08ms, ranging from 4.05ms to 5.13ms. The standard deviation for latencies using 12kHz toneburst at 92dB was +/-.46. The latency for each participant at a high intensity for a 12kHz toneburst is documented in Table 4

Table 4

Latencies at 92dB peSPL for Click and 12kHz Toneburst

Test Subject/Ear Tested	Click	12kHz
1/Right	4.50ms	4.05ms
2/Right	4.50ms	5.130ms
3/Right	5.03ms	--
4/Right	4.50ms	4.88ms
5/Right	4.65ms	Response at 102dB
6/Right	4.45ms	--
9/Right	4.65ms	4.75ms
10/Left	4.38ms	Response at 102dB

Note. "--" represents a data point unable to be determined due to no response at a high intensity. Dogs 7 and 8 were removed.

Amplitude

Click

Amplitude was determined for a click at 92dB peSPL. The average amplitude at 92dB was .38 µV with a standard deviation of +/- .16. As thresholds varied by dog, the average amplitude at threshold could not be compared. The range for amplitude at 92dB was .55µV, ranging from .16 µV to .71 µV. The amplitude of the averaged waveforms using a click at 92dB are recorded in Table 5.

12kHz Toneburst

Amplitude was also compared for a 12kHz at a high intensity. The average amplitude at 92dB peSPL was .32µV with a standard deviation of +/-.11µV. The range for amplitude at 92dB was .21µV, ranging from .22 µV to .43 µV. The amplitude for each participant at a high intensity using a 12kHz toneburst is documented in Table 5.

Table 5

Amplitudes at 92dB peSPL Using Click and 12kHz Stimulus

Subject Number	Click	12kHz
1	.41µV	.38µV
2	.35µV	.23µV
3	.16µV	--
4	.36µV	.22µV
5	.29µV	Threshold at 102
6	.71µV	--
9	.42µV	.43µV
10	.34µV	Threshold at 102

Note. "--" represents a data point unable to be determined due to no response at a high intensity. Dogs 7 and 8 were removed.

Statistical Analysis

After the *t*-test was run, it was determined there was a significant difference in the average threshold between a click and 12kHz toneburst with the average 12kHz threshold found to be higher/worse, $t(7) = -4.930$, $p = .002$, $d = -1.743$, CI(-2.853; -.591).

CHAPTER V

DISCUSSION

In this study, BAER thresholds were compared using two different types of stimuli. The purpose of this study was to identify whether a click and a 12kHz toneburst stimulus showed different hearing thresholds in older canines in order to create a BAER protocol for older dogs. Latency and amplitude were also documented to help demonstrate the rationale of the peak picking but were not statistically analyzed. It was hypothesized that thresholds would be worse when using a 12kHz toneburst compared to thresholds found using a click. The study by Ter Haar et al. (2008) demonstrated the best thresholds in young dogs were found using clicks, 12kHz tonebursts, and 16kHz tonebursts, and while threshold levels varied between Ter Haar et al. and the audibility curve (Scheifele, 2020), both demonstrated similar threshold levels between a click and 12kHz tonebursts. Thresholds collected from FETCHLAB UNC prior to this study showed thresholds were the same in a seven-week-old puppy when a click and 12kHz toneburst were used. In contrast, an aged dog might show significantly higher thresholds when using a 12kHz toneburst as opposed to a click. Additional research that contains a control group of younger dogs should be done so further comparisons between young and older dogs can be examined and impacts of age can be more clearly identified.

While a 12kHz toneburst was more likely to show a high frequency hearing loss, there are still many aspects to consider such as the purpose it would serve to find hearing loss in the high frequencies if speech understanding would not be impacted. Human studies demonstrated that presbycusis is often progressive (Lee et al., 2005) and Ter Haar et al. (2008) demonstrated a

similar progressive pattern that could eventually spread to the speech frequencies. Having this information could let us know if more frequent monitoring (either by owner or audiologist) is needed or not. Particularly in cases where presbycusis is the main concern, 12kHz toneburst testing might be a useful diagnostic tool. Findings in this study were consistent with findings in the study done by Ter Haar et al. (2008). Ter Haar et al. (2008) hypothesized that the highest frequencies would be impacted as the canines aged and their findings showed a click, 12kHz, and 16kHz had the biggest changes in threshold due to age. No analyses were completed to determine if the difference between the thresholds using the click or high frequency tonebursts were statistically significant in the Ter Haar et al. (2008) study as the point was to compare thresholds among groups. Results of the current study showed a significant difference in the average threshold for a click and 12kHz toneburst. The 12kHz toneburst was more likely to show higher thresholds than a click, and this finding suggested hearing loss was occurring in the high frequencies more than in the frequency range a click assesses. While direct comparisons should be done with caution due to the lack of a control group, thresholds taken from the seven-week-old puppy as well as findings in Ter Haar et al. (2002) and the minimal audibility curve suggested these thresholds were better in younger dogs.

Strengths and Limitations of Study

Assessing hearing in canines presents a unique set of challenges that limits the amount of useful data collected. Results are best when the subject is calm and quiet; this is not always possible with dogs. Dogs typically have a time limit in which they will tolerate testing, which means test duration and artifact are concerns when testing canines. However, the canines in this study were generally calm and quiet so participant cooperation was less of a concern. Another

challenge can be placing electrodes due to the fur canines have; however, due to the experience of the committee members, this concern was negligible.

A limitation of this study was the lack of a control group. This did not allow for threshold comparisons between age groups; however, previous findings in Ter Haar et al. (2002, 2008) suggested hearing change occurs over time as a function of presbycusis. Research in humans demonstrated that decreases in sensitivity in the high frequencies are often a result of presbycusis. Unlike Ter Haar et al. (2008) where multiple stimuli were used, only two stimuli were used in this study, which provided limited information.

A small sample size was used in this study, which could have impacted the power of the study to find an effect of aging on the different thresholds. However, this study had a high effect size of .900 when using the eight dogs, indicating the small sample size did not impact the power of this study.

Another factor that could impact testing could include the person picking the peaks for determination of threshold. This impact was avoided by having agreement on peak picking between the primary tester and the faculty research advisors. The lack of universal norms could leave more up to the tester's interpretation; however, due to the ability to have agreement on peak picking among the researchers, this concern was negligible.

During the threshold search, 20dB steps were used and once a no response was obtained, then an increase in intensity by 10dB peSPL occurred. The lowest response with a repeatable Wave V was chosen as threshold but a smaller step size could show different results. However, in order to collect all of the data needed, 10dB steps were used for the interest of time.

Implications and Future Directions

There was a statistically significant difference between click and 12kHz tonebursts that has several implications for testing canines in the future. A click is currently the most important stimulus for estimating hearing sensitivity in dogs, but in older dogs, a 12kHz toneburst might show the beginnings of age-related hearing loss where a click might not. In cases where other types of hearing loss such as a conductive, idiopathic, or congenital hearing loss are not a concern, a 12kHz toneburst should be included in testing. A click should be used to estimate hearing sensitivity in the speech frequency range, but a 12kHz toneburst could be used to determine if there are early signs of age-related hearing loss in the high frequency range. As presbycusis can be progressive, a loss seen at 12kHz might indicate the canine should be monitored whether by owner report or by additional testing at a later point.

There are several directions for future research in canines. These could include establishing norms for latencies across age groups as well as documentation of changes in middle-aged canines, which could also provide beneficial information about hearing changes across the lifespan of canines. Additional research to determine if the rate at which hearing thresholds change over time in canines could help with development of monitoring protocols. A similar study to that of Ter Haar et al. (2002) measuring thresholds, latencies, and amplitudes using multiple stimuli would be beneficial for older and middle-aged dogs. It was observed that the older participants in the current study had higher overall thresholds, and future research could examine changes in thresholds across smaller age ranges. Amplitude, latency, and thresholds using stimuli types should also be examined across age groups in depth.

Conclusions

Canines are thought to have progressive hearing loss due to aging similar to that reported for humans, which is first seen in the frequency range above 8kHz. Findings in past studies of canines demonstrated a pattern of hearing loss that could be progressive and typically starts in the higher frequencies (Ter Haar et al., 2002, 2008, 2009). Currently, in canine auditory testing, a click is the most commonly used stimulus as it gives a good estimate on hearing thresholds in the speech range; however, a click might miss the characteristic high frequency hearing loss of presbycusis that a high frequency toneburst might show. The purpose of this study was to determine if there was a difference in the thresholds for a click and a high frequency toneburst. It is important to remember, however, that BAER test results do not establish the presence or absence of age-related hearing loss. It is simply a predictor of possible decline in auditory responses.

Dogs nine years and older were tested using both stimuli and after the analysis, a statistically significant difference between thresholds for the 12kHz and click stimulus was found with 12kHz having overall worse thresholds. This indicated 12kHz was better at detecting high frequency losses than a click. Due to the limited amount of data, it was unclear from this study if and how these thresholds changed over time; however, Ter Haar et al. (2008) found evidence to suggest these changes are often progressive. While further research is needed to understand age and canine hearing, this suggested a click and high frequency toneburst such as the 12kHz toneburst could be useful screening tools to monitor for progressive changes to hearing. While a click might be useful functionally as it assesses hearing closer to the range of human speech, it might miss the beginnings of presbycusis that can present as a progressive loss (Ter Haar et al., 2008). Based on these results, the protocol for older dogs should include both a 12kHz toneburst

and a click, particularly when the click thresholds indicate normal or near normal hearing sensitivity. Using a click would allow for the canine's owner to have more information about how their dog hears their voice but a 12kHz toneburst could indicate further monitoring is needed if the threshold is higher than expected. Hearing care professionals should also do a clear case history to determine what concerns there are. For example, a 12kHz toneburst should be used in conjunction with a click, particularly when concerns are focused on age-related hearing loss as a high frequency toneburst threshold search might detect a progressive decline in auditory responses, which could in turn impact counseling of owners and future testing recommendations.

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APPENDIX A

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL

UNIVERSITY OF
NORTHERN COLORADO

Institutional Animal Care and Use Committee

Date: December 22, 2020

Principal Investigator: Dr. Tina Stoody

Committee Action: **IACUC Protocol- New Protocol Approval**
Action Date: December 22, 2020

Protocol Number: 2009C-TS-D-23
Protocol Title: Extended High Frequency BAER Testing in Aging Canines
Expiration Date: December 22, 2023

The University of Northern Colorado Institutional Animal Care and Use Committee (IACUC) APPROVED animal use protocol, **Extended High Frequency BAER Testing in Aging Canines – 2009C-TS-D-23,** on December 22, 2020 for a three-year approval period.

The committee's review was based on the requirements of the Government Principles, Public Health Policy, USDA Animal Welfare Act and Regulations, the Guide for the Care and Use of Laboratory Animals, as well as university policies and procedures related to the care and use of animals at the UNC. Based on the review, the IACUC has determined that all review criteria have been adequately addressed. The PI is approved to perform the experiments or procedures as described in the protocol as approved by the committee. It is the responsibility of the PI to be familiar with and comply with the protocol and all pertinent institutional, state, and federal rules and policies. The PI must confirm and document that all personnel complete the required training in the care and use of laboratory animals and acquire specific training in all assigned procedures prior to beginning their work on this protocol.

During the three-year approval period, annual IACUC review of the protocol is required for animal use to continue. These annual reviews, known as Continuations, must be submitted by the Principal Investigator on or before the anniversary date of the initial approval date noted above. To continue this research beyond the three-year approval period, a new protocol submission is required for IACUC review. To avoid a lapse in IACUC approval, it is essential that the

completed protocol be submitted and approved by the IACUC prior to the expiration date noted above.

It is also the responsibility of the Principal Investigator to notify the IACUC of any:

- Proposed changes regarding the work described within this protocol. The PI agrees that no such changes will be implemented until an amendment has been approved by the IACUC or is encompassed under veterinary care.
- The IACUC regarding any adverse events or unexpected study results that impact the animals or personnel. Any unanticipated pain or distress, morbidity or mortality must be immediately reported to the attending veterinarian and the Director of Compliance and Operations, ACUP.

If you have any questions, please contact the UNC Animal Care and Use Program (ACUP) Director, Laura Martin, at 734-730-6631 or via e-mail at laura.martin@unco.edu. Additional information concerning the requirements for the welfare and use of animal subjects can be found at the websites for the University of Northern Colorado ACUP https://www.unco.edu/research/research-integrity-and-compliance/iacuc/, the NIH's Office of Laboratory Animal Welfare https://olaw.nih.gov/, and the USDA's Animal Plant and Health Inspection Services https://www.aphis.usda.gov/aphis/home/.

Sincerely,

Laura W. Martin
Director of Compliance and Operations
Animal Care and Use Program

OLAW Assurance: D16-00579
USDA Registration: 84-R-0008

APPENDIX B

OWNER OF PARTICIPANT CONSENT FORM

Project Title: Extended High Frequency BAER testing in aging canines

Principal Investigators: Rebecca Arnold, B.S., & Tina Stoody, PhD.
Contact Phone Number: (630) 636- 0577
Contact email: arno8016@bears.unco.edu
Faculty Advisor: Tina Stoody, PhD

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

We will be using very tiny, subdermal needles electrodes placed in three (3) different locations on the dog. Lidocaine/Prilocaine (2.5%/2.5%) will be applied to these locations, before placing the electrodes, to numb the area. Foam insert earphones will be inserted into the ear canal of the ear being tested, and a click or tone will be presented. This protocol may take up to 30 minutes, but typically lasts no more than 5-10 minutes.

The staff on site will provide a brief wellness examination of your dog before the BAER test, that will look at your dog's temperature, respiration, heart rate, etc. Members of the research team will gentle restrain your dog. The test requires the dog to stay relatively still, so consult with your veterinarian regarding the necessity for sedation. You will receive a report of the state on the state of your dog's auditory health and any recommended follow-up activities. All hearing screenings/assessments will be analyzed and confirmed by an audiologist and the principal investigator graduate student.

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

Dog Owner	**Principal Investigator**
Signed: _____	**Signed: _____**
Name: _____	**Name: _____**
Date: _____	**Date: _____**

APPENDIX C

PRE-RECORDED CHECKLIST

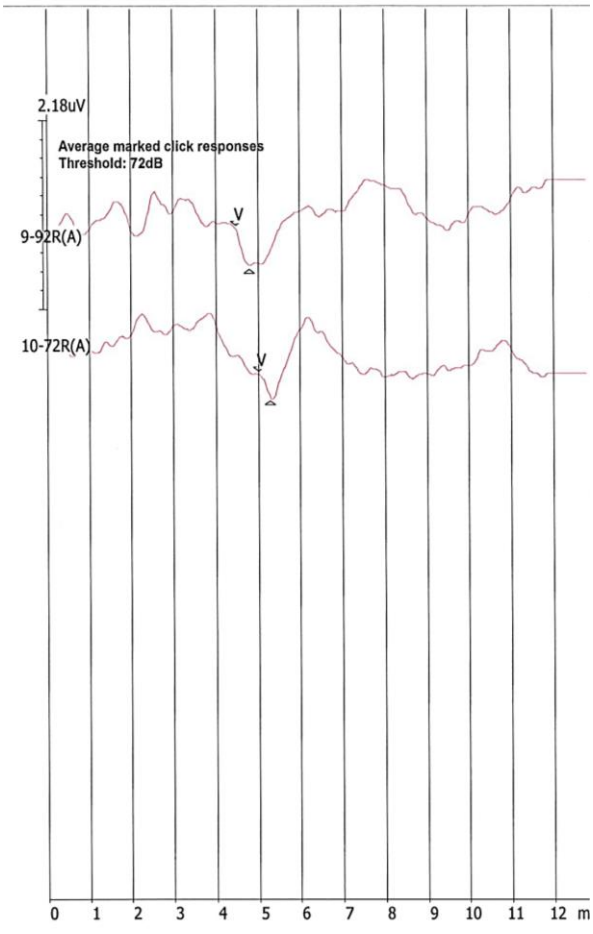
BEFORE ARRIVAL/TESTING: Verify that the dog is up to date on vaccines, especially rabies.

1. Consent Form
2. GAs will bring dog in from the owner's car – per COVID restrictions, the owners must remain in car for duration of testing
3. Health Check
4. Thundershirt placement
5. Lidocaine/Prilocaine Placement
6. Add participant to computer system
7. Subdermal needle placement
8. Impedance check
9. Alternating Polarity
10. 33.3 clicks/sec
11. 1,000 sweeps
12. Filter: 100-1500Hz
13. Absolute gain of 100,000
14. Artifact rejection rate of 35.1%
15. Insert in more accessible ear
16. Click at 92dB peSPL; if no response go up to 102dB
    a. Go down by 20dB until no identifiable/repeatable wave V
    b. Go up 10dB and run 2 sweeps
17. 12kHz at 92dB peSPL; if no response go up to 102dB
    a. Go down by 20dB until no identifiable/repeatable wave V
    b. Go up 10dB and run 2 sweeps
18. Save
19. Unhook dog and give treat
20. Return dog to owner in car

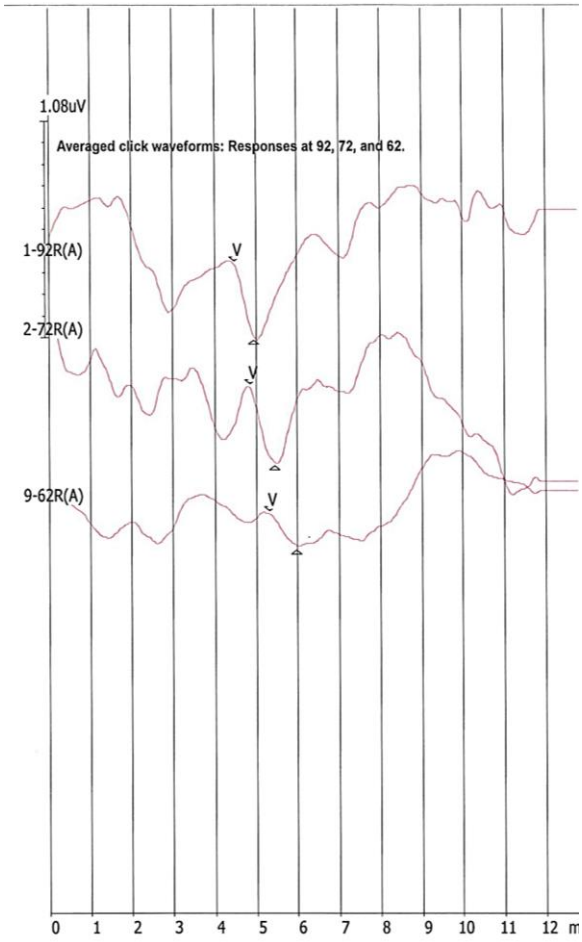
APPENDIX D

AVERAGED CLICK WAVES

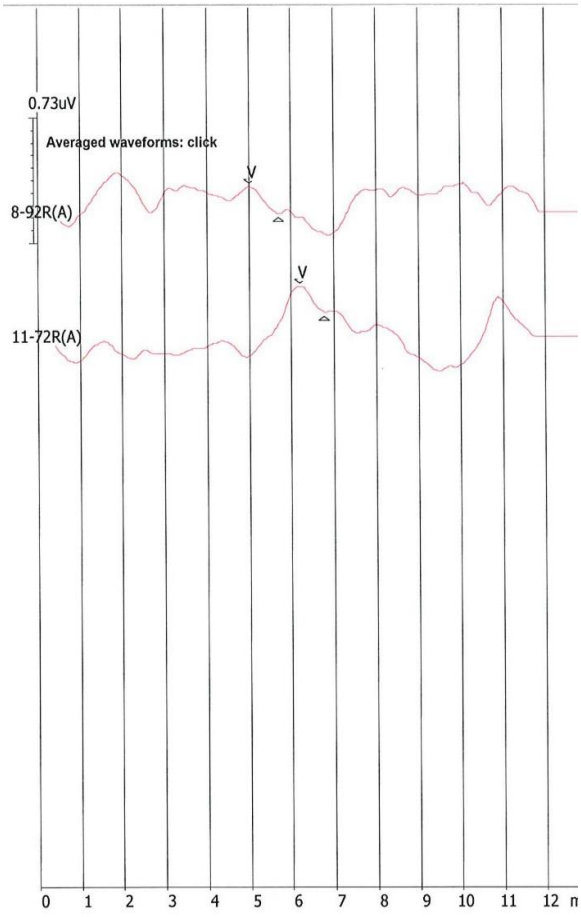
Participant 1: Averaged click thresholds



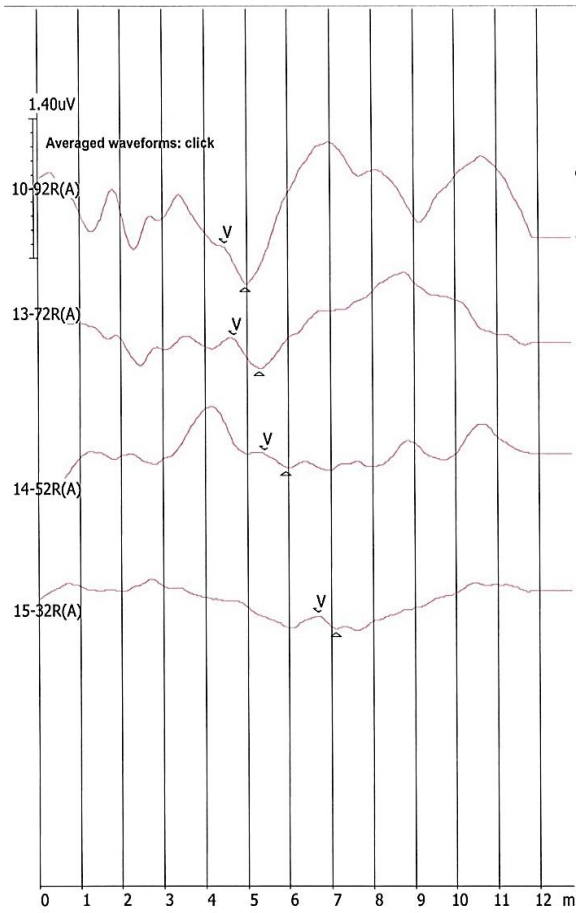
Participant 2: Averaged click thresholds



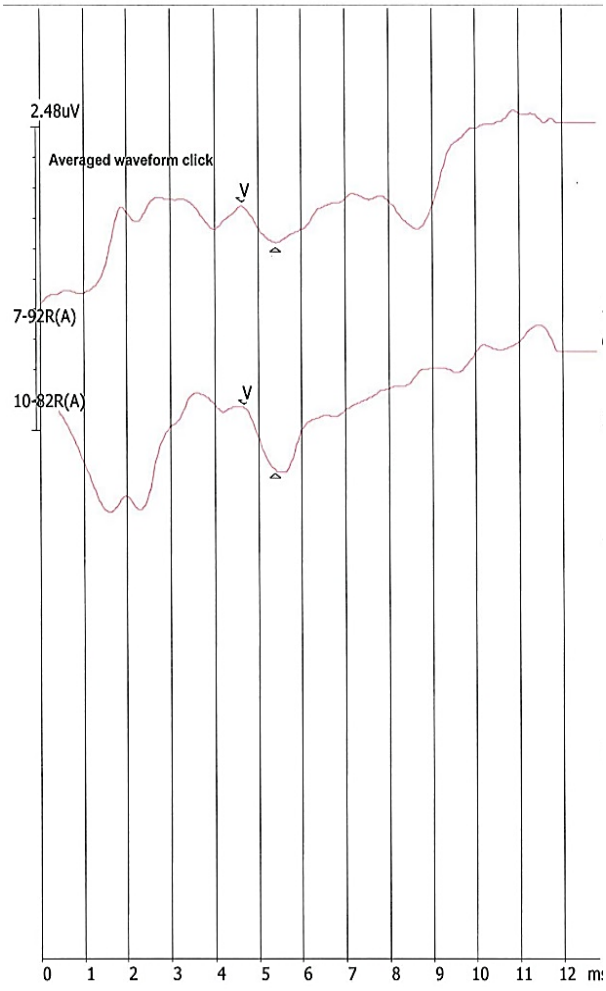
Participant 3: Averaged Click Thresholds



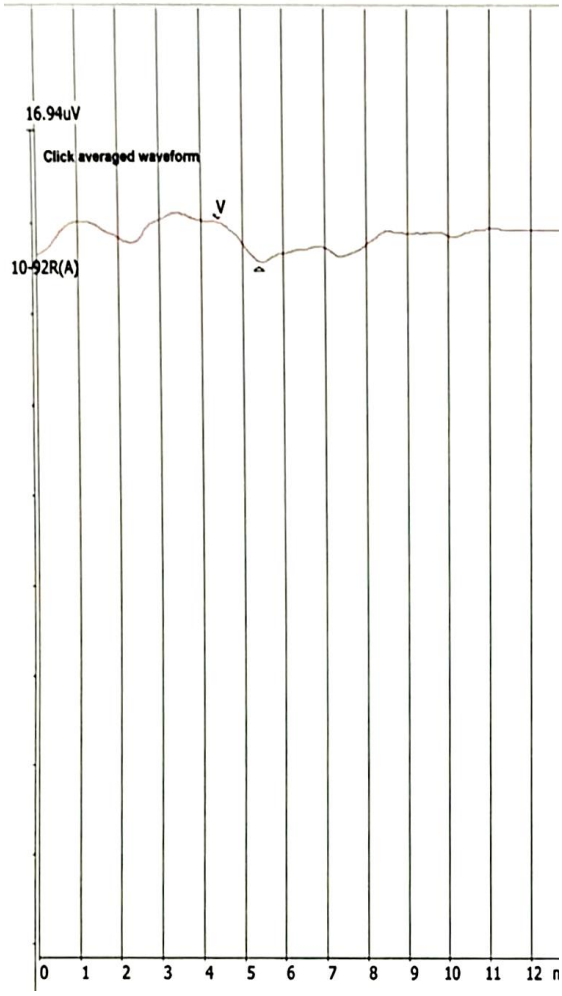
Participant 4: Averaged Click Thresholds



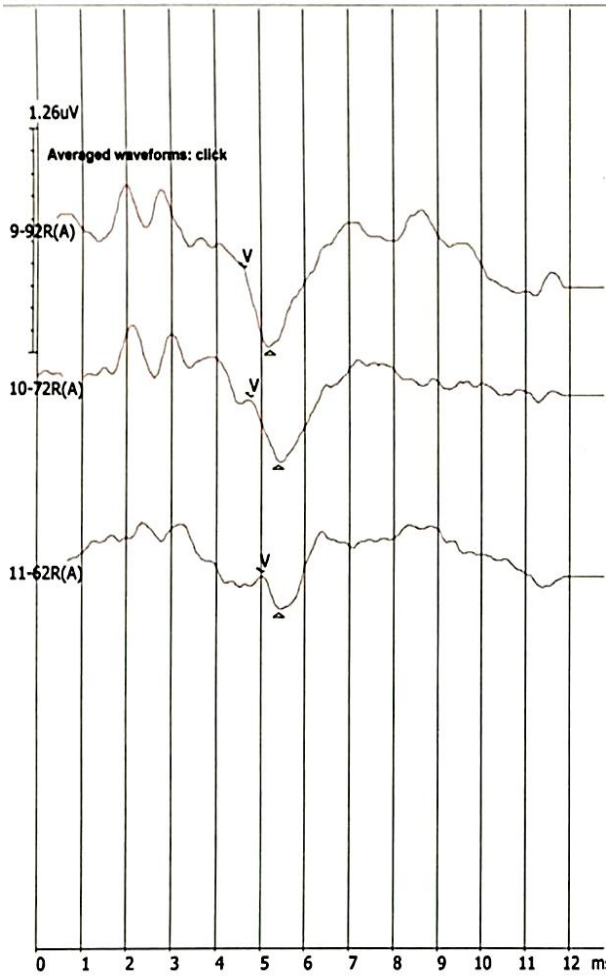
Participant 5: Averaged Click Threshold



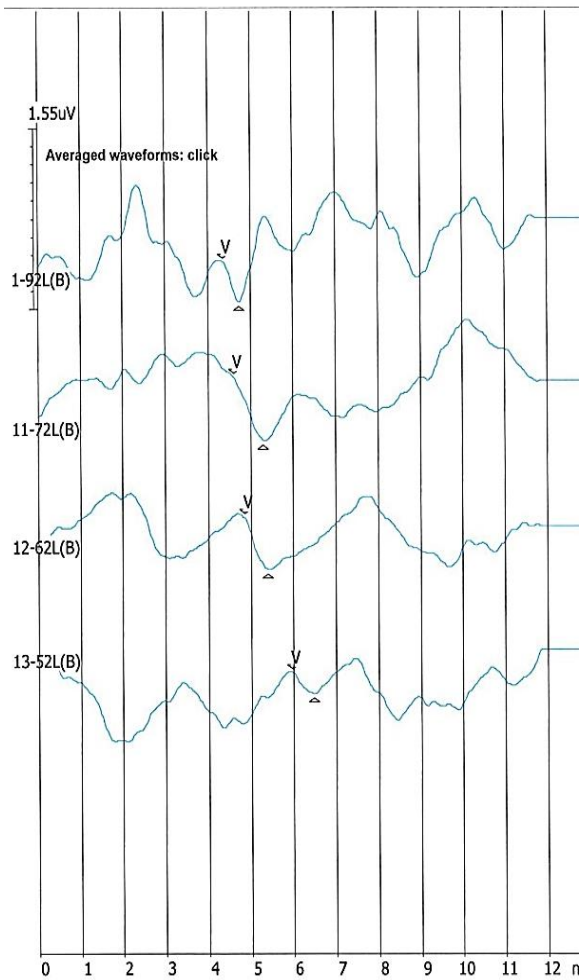
Participant 6: Averaged Click Threshold



Participant 9: Averaged Click Threshold



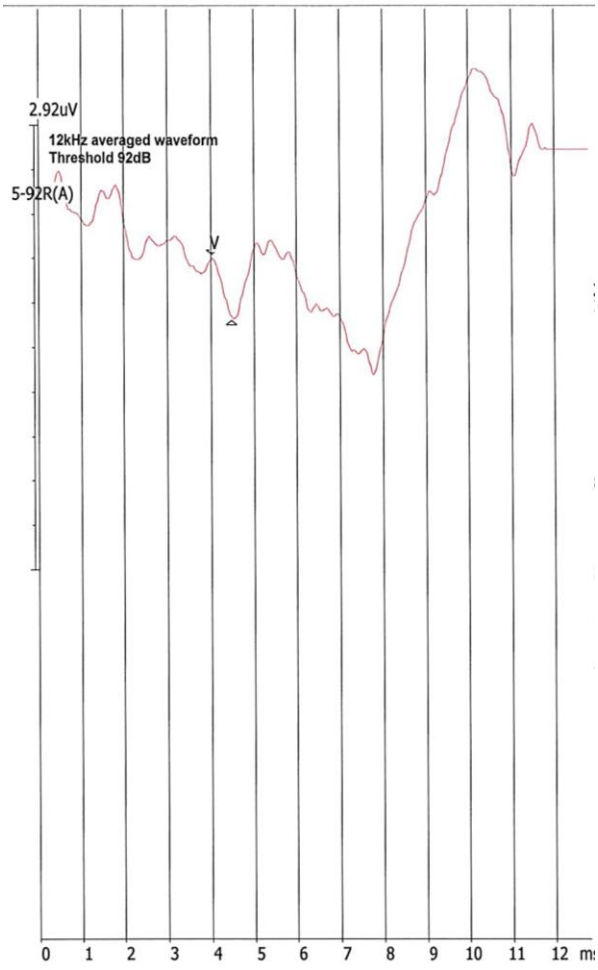
Participant 10: Averaged Click Threshold



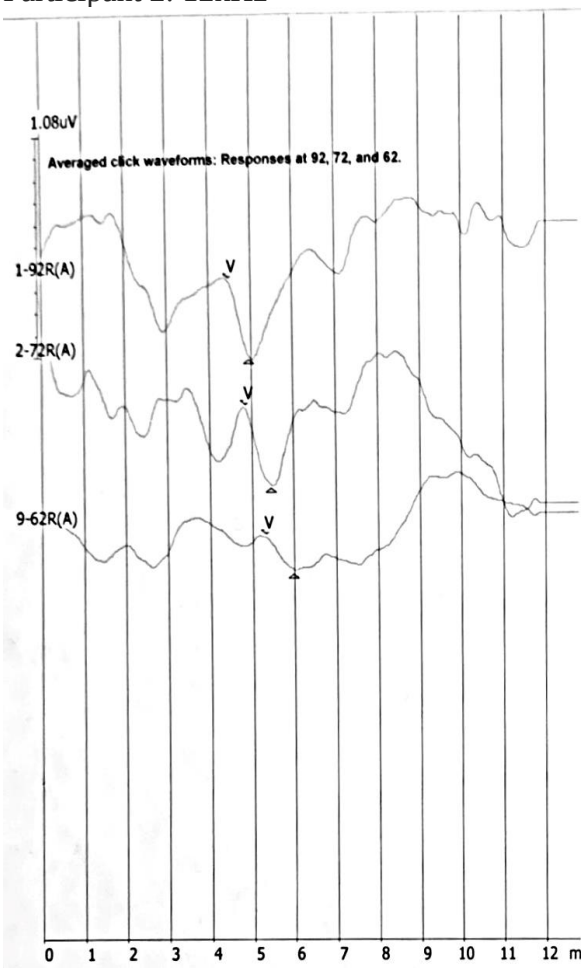
APPENDIX E

12kHz TONEBURST AVERAGED WAVEFORMS

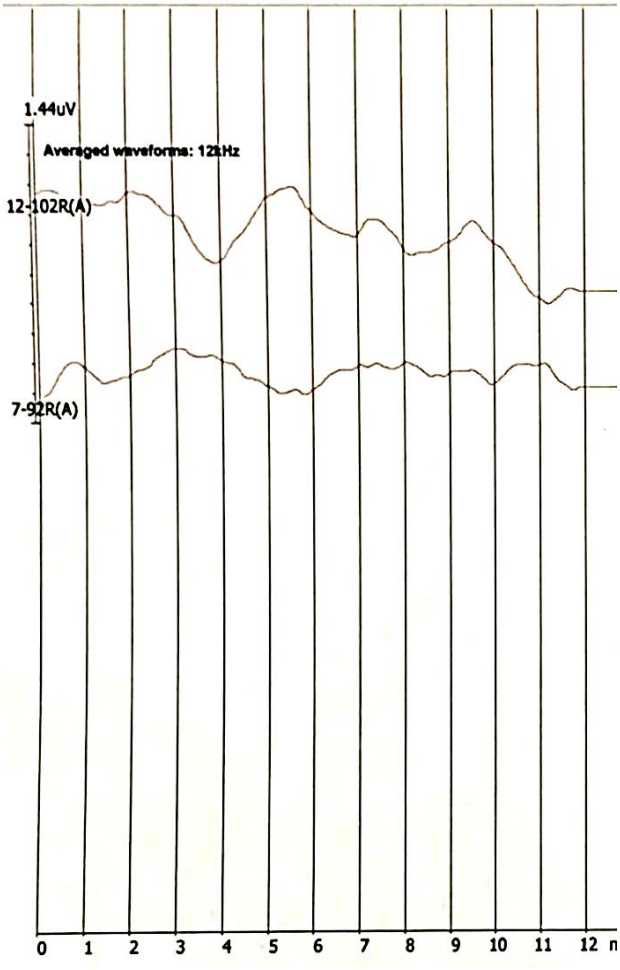
Participant 1: 12kHz toneburst



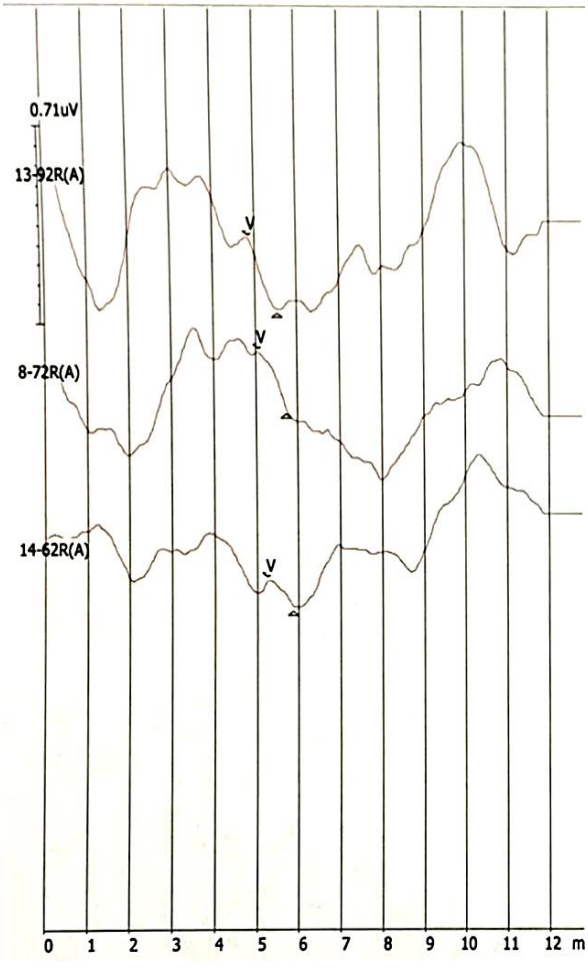
Participant 2: 12kHz



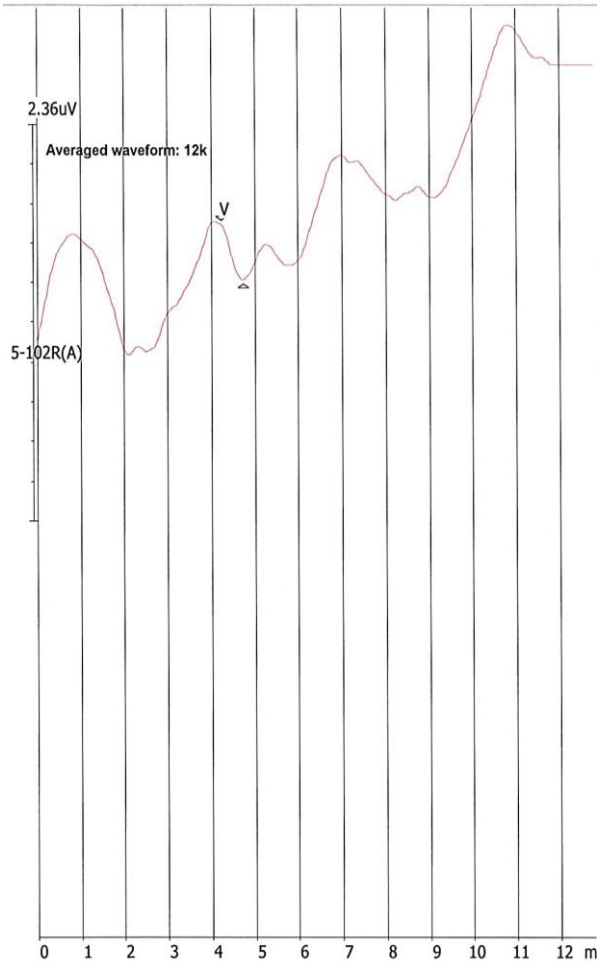
Participant 3: 12kHz



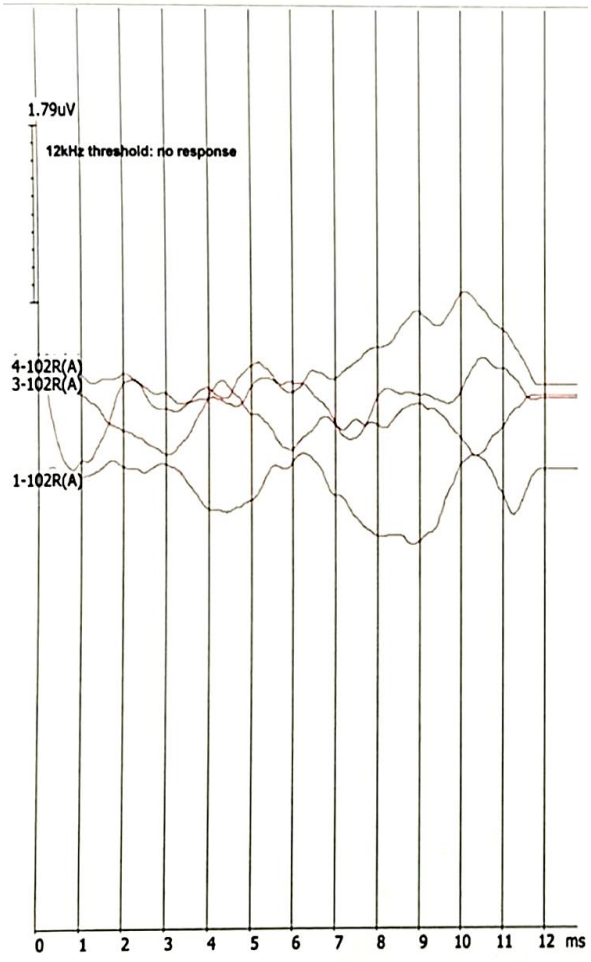
Participant 4: 12kHz



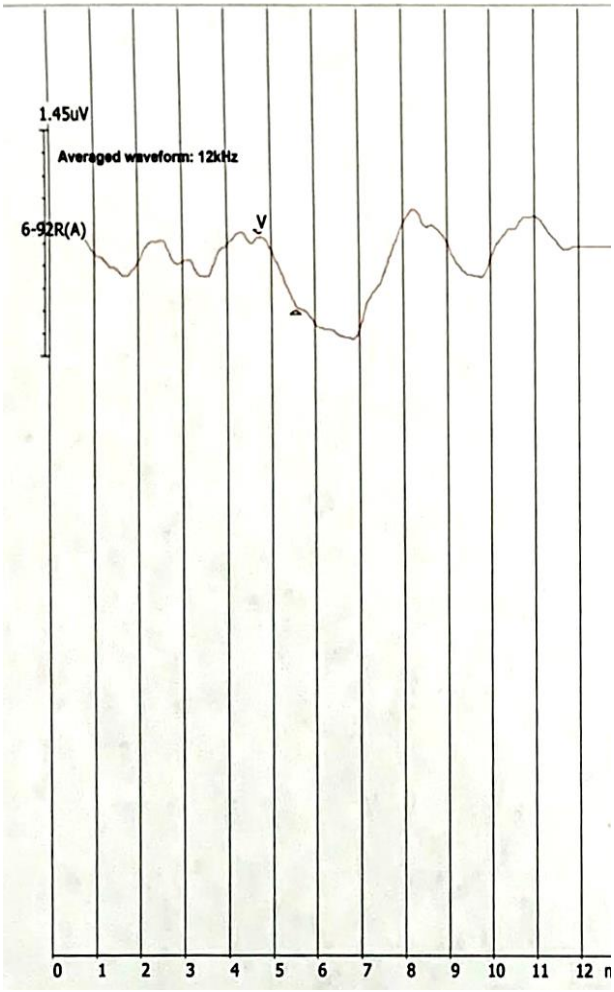
Participant 5: 12kHz



Participant 6: 12kHz



Participant 9: 12kHz



Participant 10: 12kHz