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Distortion product otoacoustic emissions in canines: Systematic Changes in amplitude as a function of f2/f1 ratio

Sara Hartson
hart7144@bears.unco.edu

UNIVERSITY OF NORTHERN COLORADO

Greeley, Colorado

The Graduate School

DISTORTION PRODUCT OTOACOUSTIC EMISSIONS IN CANINES: SYSTEMATIC CHANGES IN AMPLITUDE AS A FUNCTION OF F2/F1 RATIO

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Sara Hartson

College of Natural and Health Sciences
Department of Audiology & Speech-Language Sciences

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Accepted by the Scholarly Project Research Committee

____

Kathryn Bright, Ph.D., Co-Research Advisor

____

Jennifer E. Weber, Au.D., Co-Research Advisor

____

Tina M. Stoody, Ph.D., Committee Member

Accepted by the Graduate School

____

Jeri-Anne Lyons, Ph.D.
Dean of the Graduate School
Associate Vice President for Research

ABSTRACT

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Distortion product otoacoustic emissions (DPOAEs) are part of hearing screening measures as well as the comprehensive audiologic test battery in humans. For canines, the brainstem auditory evoked response (BAER) test is currently the gold standard for assessing hearing sensitivity in canines. This procedure is minimally invasive, time intensive, costly, and does not provide frequency specific information when using a click stimulus. This could create challenges in accessibility and ability to test canines' hearing status on a large scale. Distortion product otoacoustic emissions can be a quick, beneficial, and noninvasive method for providing ear-specific information on cochlear function of a canine. Performing DPOAEs on canines for screening and/or diagnostic purposes is a relatively new area of research and there is currently a lack of canine-specific testing equipment, settings, protocols, and universally accepted clinical normative data and guidelines. The purpose of this research was to determine what stimulus frequency ratios would produce the most robust DPOAEs in canines to support future clinical use of DPOAEs to aid in evaluation of hearing status in canines.

Diagnostic DPOAE tests utilizing nine f2/f1 ratios in the range of 1.18-1.28 were completed on the right ear of 10 canine subjects with Mutt Muffs® (Safe & Sound Pets, 2021) securely covering the test ear. Results from all 10 subjects showed consistent DPOAE responses across the different f2/f1 ratios tested. Therefore, it could be concluded that DPOAEs could be elicited from the canine ear. The f2/f1 ratios between 1.18 and 1.28 did not produce statistically

significantly different DPOAE responses and the pattern across frequencies did not significantly change when the f2/f1 ratio changed. Results from the study also showed the typical canine DPOAE responses were observed at 2k Hz and above. Lack of DPOAE responses below 2k Hz regardless of the noise floor levels, low ambient noise, and level of canine compliance, and degree of quietness suggested the typical DPOAE response was best at 2k Hz and above. In general, canine DPOAE responses were robust enough that the f2/f1 ratio did not seem to significantly impact the overall amplitudes. Due to the lack of a probe specifically designed for canine ears, use of Mutt Muffs or a similar device is recommended, although further research is necessary to determine if resonance under the Mutt Muffs changed responses before DPOAEs could be incorporated into veterinary practices as a method for hearing screenings/diagnostic purposes. If DPOAEs are to be utilized as a clinical screening tool at this time, a f2/f1 ratio of 1.18-1.22 is recommended. The f2/f1 ratios 1.18 and 1.20 had the most robust responses overall; therefore, it would be ideal to use ratios within that range. The ratio 1.22 is typically used for DPOAEs in humans and due to the responses also being robust at this f2/f1 ratio, it would be acceptable to use 1.22 at this time especially if it aided in ease of use.

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CHAPTER I

STATEMENT OF THE PROBLEM

Clinical assessment of hearing sensitivity of canines is a relatively new topic of research and only a limited amount of research has been conducted on best practices for hearing sensitivity measurements, result interpretations, and guidelines. Currently, the brainstem auditory evoked response (BAER) test is the only hearing sensitivity assessment accepted by the Orthopedic Foundation for Animals (Sims et al., 2017). This procedure is minimally invasive as it only requires small needle electrodes to be placed underneath the skin in three to four locations on the canine's head. The BAER test provides ear specific information and testing can be performed at multiple intensity levels and/or with different stimulus frequencies for a diagnostic evaluation or threshold estimation measure. The time, cost, and use of needle electrodes can create challenges in access and ability to test canines' hearing status on a large scale.

Otoacoustic emissions (OAEs) can be a beneficial and noninvasive method for providing ear-specific information on the hearing status of a canine. Evaluations of OAEs have been completed for canines with respect to age, breed, size, gender, level of consciousness, and status of hearing sensitivity; however, there is still more to learn about canine OAE responses. For diagnostic and screening purposes, it is imperative that universally accepted clinical normative data and guidelines be determined. There is no current reference for appropriate settings to collect accurate and robust OAEs in canines. There are no guidelines for what constitutes a typical or atypical response with respect to OAEs in canines. Additionally, there has been no consensus on a universally accepted probe tip design to reduce ambient noise. With this lack of data, OAEs have not been widely accepted as a measure to evaluate hearing status in canines. To perform and interpret OAE or BAER tests on canines, veterinarians and/or audiologists need to have knowledge of testing parameters and procedures as well as be trained in result interpretation for accurate diagnoses.

There are differences and similarities between canine and human auditory systems. The canine cochlea has more turns than a human cochlea (West, 1985); therefore, it could be inferred that canine basilar membranes would be longer and have a greater range of audible frequencies than humans (Strain, 2012). Prior to using OAEs clinically to aid in diagnosis of hearing status in canines, research needs to be conducted to determine testing procedures, equipment modifications, and software settings that would produce optimal OAEs in canines. Therefore, the purpose of this study was to determine what stimulus frequency ratios produced the most robust OAEs in canines to support future clinical use of OAEs to aid in diagnosis of hearing status in canines.

Research Questions

Q1 When measuring distortion product otoacoustic emissions (DPOAEs) in canines, does the amplitude pattern across frequencies for the 2f1-f2 distortion product change with changes in the f2/f1 ratio?

Q2 When measuring distortion product otoacoustic emissions (DPOAEs) in canines, do specific f2/f1 ratios produce different overall amplitudes of the 2f1-f2 emission?

Hypotheses

H1 The amplitude patterns across frequencies for the 2f1-f2 distortion product will vary with changes in f2/f1 ratio for canine DPOAEs.

H2 Changing the f2/f1 ratio produces different overall amplitudes of the 2f1-f2 emission.

CHAPTER II

REVIEW OF THE LITERATURE

The Canine Auditory System

Canine auditory systems have structural similarities and differences when compared to the auditory system of humans. Moving medially, the anatomical configuration of the canine ear is similar to the human ear, starting with the pinna and moving to the ear canal, tympanic membrane, middle ear, and inner ear. The canine pinna consists of cartilage and can be either erect or pendulous (Njaa et al., 2012). Erect pinnae stand up and pendulous pinnae hang down the side of the canine's head. The purpose of the pinna is to be mobile so it can assist in localization, collect the sound waves, and transmit them down the canal toward the tympanic membrane (Cole, 2009; Njaa et al., 2012). The external canal opening faces dorsolaterally and the tragus forms the boundary of the ear canal laterally (Njaa et al., 2012). The antitragus is separated from the tragus via the intertragic incisure, which many clinicians use to aid in guiding the otoscope into the ear canal to perform otoscopy on the canine (Njaa et al., 2012). The ear canal has two portions: the vertical ear canal, which forms from the concha, and the horizontal ear canal, which leads to the tympanic membrane. At the point where the vertical canal turns into the horizontal canal, there is a ridge, referred to by some using the informal anatomical term *Noxon's Ridge*, which can make direct access to the tympanic membrane difficult. To properly insert the otoscope and the probe, the pinna must be pulled up and back. Canines have hair in the outer portion of the ear canal that decreases when moving medially toward the tympanic membrane. Cerumen, or ear wax, is produced through the sebaceous and ceruminous glands in the ear canal.

The tympanic membrane is a tri-layered semitransparent membrane that separates the outer ear from the middle ear. In canines, the tympanic membrane is angled at 45-degrees relative to the horizontal ear canal (Njaa et al., 2012). It has a small upper section termed the pars flaccida that is usually flat; however, if it is bulging, that is not necessarily abnormal. A bulging pars flaccida can be normal but it can sometimes indicate otitis media. The tympanic membrane also has a lower section named the pars tensa, which is larger, thinner, tougher, and consists of strands that radiate outward (Cole, 2009). The malleus is the most lateral of the middle ear ossicles and attaches to the tympanic membrane via the manubrium (head). This causes the tympanic membrane to have a concave shape when viewed through an otoscope. The function of the tympanic membrane is to vibrate from the sound-generated waves of air pressure and transfer the energy from the vibrations to the ossicles in the middle ear. This is a transformation of acoustic energy to mechanical energy.

The middle ear anatomy of canines is similar to that of humans; however, some differences are worth noting. The middle ear is an air-filled cavity with three ossicles (malleus, incus, stapes) suspended through it. The middle ear cavity has three sections: the epitympanic recess, ventral cavity, and tympanic cavity proper. The epitympanic recess is the small space that holds the malleus and incus (Cole, 2009). The tympanic cavity proper is adjacent to the tympanic membrane and has an opening that goes to the ventral cavity. The ventral cavity is the largest of the three sections (Cole, 2009). The middle ear ossicles function to transfer the energy from tympanic membrane to the oval window in the inner ear, transforming the mechanical energy into hydro-mechanical energy since the inner ear is filled with fluid. As is the case in humans, the structures of the middle ear also function to overcome the impedance mismatch that occurs at this point because fluid has a higher impedance than air, so it takes more energy to push the sound through fluid. The auditory system accounts for the mismatch in two ways: (a) the tympanic membrane surface area is much larger than the oval window surface area, and (b) the incus and malleus are constructed to act as a lever system. These two methods work to amplify the sound pressure wave to overcome the impedance mismatch that occurs when moving the energy from air through fluid (Njaa et al., 2012). The Eustachian tube in canines extends from the tympanic cavity proper in the middle ear cavity to the nasopharynx. The Eustachian tube has the same pressure equalization function in the middle ear of canines as it does in humans (Cole, 2009).

Continuing to move medially through the canine auditory system, the inner ear is located in the petrous section of the temporal bone of the canine (Cole, 2009). The middle ear connects to the inner ear via the oval window and the round window. The stapes footplate fits into the oval window and when the sound is transferred through the ossicles, the footplate transforms the energy to the fluid in the inner ear. The inner ear consists of the vestibule, three semicircular canals, and a cochlea that is spirally shaped. The canine inner ear has two main functions: to detect head movement and to detect sound. The utricle and saccule are responsible for detecting linear movement of the head and its position when static. The semicircular canals also detect movement from head position rotation. The cochlea is responsible for sound detection (Cole, 2009). The coil of the cochlea is filled with fluid and is separated into different compartments called the scala vestibuli and the scala tympani. In between these two compartments is the scala media, which is separated from the scala vestibuli and the scala tympani by Reissner's membrane and the basilar membrane, respectively. The organ of Corti, the sensory auditory organ, sits on the basilar membrane. The cells on the organ of Corti are called hair cells and they move when the fluid above them moves (as a result of sound energy being transferred throughout the auditory system). The hair cells transmit the information they receive from the movement, which are afferent and efferent innervations from the cochlear branch of cranial nerve VIII (Njaa et al., 2012). This sends the signals to the brain to be processed as sound. The functions of the canine inner ear are similar to the functions of the inner ear in humans.

For canines, it was reported by Cole (2009) that responsiveness to sound begins at day 14 on average after birth. The most utilized way to measure the responses to sound in canines is through auditory evoked responses. The method used currently is called the BAER test. Analysis of wave peaks I, II, III, and V, wave latencies, interpeak latencies, peak amplitudes, and thresholds help to determine hearing status in canines. It was found that by day 40, canines reached adult values of the BAER test (Cole, 2009).

Canine's hearing sensitivity does differ from human's hearing sensitivity in several ways. The frequency range audible to canines is approximately 67 Hz to 45k Hz according to Strain (2012), which is a much greater range than humans (approximately 67 Hz to 23k Hz). Lipman and Grassi (1942) found that although the hearing sensitivity of canines was comparable to that of humans in the frequency range of 125-250 Hz, above 250 Hz canines had superior hearing sensitivity than humans. According to West (1985), another difference between canine and human auditory systems is canine cochleas have approximately 3¼ turns whereas human cochleas have on average 2¾ turns. West reported that the number of turns in the cochlea is related to the range of audible frequencies and basilar membrane length is related to the limits of hearing on each end of the range. Increasing the number of turns in the cochlea could potentially increase the octave range the mammal can hear (West, 1985), which is illustrated through the fact that canines have more turns in their cochleas than humans and they have a larger range of audible frequencies. Although there are anatomical and physiological aspects of canine auditory system that are similar to the human auditory system, the differences mentioned above are important to note as they can alter hearing sensitivity.

Types of Hearing Loss in Canines

Conductive Hearing Loss

Conductive hearing loss occurs when a pathology is present in the outer and/or middle ear that prevents sound from traveling to the inner ear, which still functions adequately. The prevention of sound from traveling through the outer ear might be due to foreign objects, excessive cerumen blocking the ear canal, otitis externa (infection located in the ear canal), inflammation of the ear canal, or atresia (narrowing or lack of ear canal opening; Strain, 2012). Middle ear pathologies that might cause a conductive hearing loss are otitis media/primary secretory otitis media (infection in the middle ear), middle ear polyps, and potentially otosclerosis (Strain, 2012). A majority of the causes of conductive hearing loss are able to be fixed or treated through surgery, medication, or removal. If the inner ear is functioning normally and the outer or middle ear pathology has been corrected, then the canine’s hearing would be within normal limits.

Sensorineural Hearing Loss

Sensorineural hearing loss occurs when a pathology is present that impacts the function of the hair cells and/or neurons in the cochlea and/or the vestibulocochlear nerve (CN VIII). Some causes of sensorineural hearing loss are congenital: pigment-associated and non-pigment-associated hearing loss, perinatal anoxia, dystocia, and intrauterine ototoxicity exposure (Strain, 2012). Other causes of sensorineural hearing loss include ototoxicity (exposure to drugs/chemicals that are toxic to the inner ear), otitis interna (infection in inner ear), noise-induced hearing loss, presbycusis (age-related hearing loss), trauma, anesthesia-associated hearing loss, or hearing loss due to unknown cause(s; Strain, 2012). At this time, sensorineural hearing loss in canines cannot be corrected. Damage to the hair cells, neurons, or vestibulocochlear nerve is permanent most of the time.

Distortion Product Otoacoustic Emissions Testing

Otoacoustic emissions (OAE) testing is not a test of hearing sensitivity; it is an objective test of cochlear function. Evoked OAEs are emissions produced as a result of an acoustic stimulus. The outer hair cells in the cochlea respond specifically to the sound produced in the ear, which evokes a specific response that can be measured. The specific response of outer hair cells is referred to as outer hair cell motility, which is the elongation and compression of outer hair cells in response to sound stimulation. This motility along with the movement of the basilar membrane causes a cochlear amplifier effect. The cochlear amplifier effect is the location on the basilar membrane where there is a boost of energy at the place of maximum displacement when sound stimulation causes a forward traveling wave. Traveling waves always move base to apex because of the width of the basilar membrane (base thinner than apex) and the stiffness gradient (base stiffer than apex), which means the high frequencies at the base always respond first and low frequencies at the apex take longer to respond. For every frequency, there is a specific place of maximum displacement, meaning the location of the peak is dependent on frequency (Dallos, 1988). Only healthy ears have the cochlear amplifier effect, which is responsible for exquisite hearing ability to differentiate frequencies and improve the dynamic range. When the ear is oxygen deprived or experiences damage, the outer hair cells are lost first, resulting in the loss of the cochlear amplifier effect. Because the outer hair cells are responsible for the first 50-60 dB of hearing, when damage occurs that causes loss of outer hair cells, the individual experiences approximately 50-60 dB of hearing loss and reduced frequency resolution due to the loss of the cochlear amplifier effect. The method for measuring the cochlear amplifier effect clinically is through OAEs. The OAEs are a direct measure of the motility of outer hair cells. External sound sources cause forward traveling waves toward the apical end of the cochlea in addition to reverse traveling waves that travel back toward the basal end of the cochlea, back through the middle ear to re-excite the tympanic membrane, and produce tiny sounds/emissions in the ear canal (Kemp, 1986). The forward traveling wave at the place of maximum displacement on the basilar membrane causes a process to occur where the metabolic energy pool feeds the energy of the vibrations to travel backwards in what is called a backward traveling wave (Dallos, 1988). The pathway consists of the energy flow moving forward through the auditory system; then at the outer hair cell location, it reverses and flows back out to the external ear and acoustic environment (Dallos, 1988).

There are different types of evoked otoacoustic emissions testing, one of which is called distortion product otoacoustic emissions (DPOAE) testing. "DPOAEs actually comprise a mixture of backward-traveling waves that arise by two fundamentally different mechanisms within the cochlea" (Shera & Guinan, 1999, p. 791). Otoacoustic emissions emerge from two mechanisms: the source of distortion near f2 and coherent reflection near the distortion product frequency (Shera & Guinan, 1999). Distortion products are the vector sum of two components generated from the nonlinear distortion as well as the linear (coherent) reflection (Botti et al., 2016; Shera & Guinan, 1999; Zelle et al., 2015). Linear (coherent) reflections are backward-traveling waves that occur as a result of cochlear anatomy and mechanics producing random impedance perturbations from the forward-traveling waves (Shera & Guinan, 1999). Nonlinear distortion is where the two-tone stimulus causes a nonlinear response by the cochlea. The forward-traveling waves create spatial distortions that become the sources of the backward-traveling waves (Shera & Guinan, 1999).

Otoacoustic emissions testing provides insight into how the outer hair cells in the cochlea are functioning. The test is ear specific as it tests each ear individually and results are shown for each ear. It is not a direct test of hearing sensitivity; however, research has shown relationships between DPOAE measures and hearing status. As a result, DPOAE testing can be used for screening or diagnostic purposes. A quick screening can be performed to get a basic idea of the status of overall cochlear function. A more in-depth diagnostic test can provide specific information regarding the functioning ability of the cochlea as well as provide indications for hearing status. Gorga et al. (1997) found DPOAEs accurately corresponded to hearing status for the 1500-6000 Hz frequency range with slightly less accurate agreement to the audiogram for frequencies above and below that range. It was shown that DPOAE measures had a relationship to audiometric thresholds where hearing losses less than 60 dB HL could be accurately identified through DPOAEs (Gorga et al., 1997).

To perform evoked OAE testing, a probe that contains a microphone is fit into the ear canal of the patient. The probe presents a stimulus (two pure tones for DPOAEs), which causes a healthy cochlea to produce OAEs in response to the stimulus. The emissions travel back from the cochlea to the ear canal where they are measured and recorded by the probe microphone and displayed on a computer system. Distortion product otoacoustic emissions are commonly used in clinical settings to assess cochlear function (Abdala & Visser-Dumont, 2001; Gorga et al., 1997; Scheifele & Clark, 2012; Shera & Guinan, 1999; Zelle et al., 2015).

Distortion product otoacoustic emissions are OAEs evoked in response to two tones at different frequencies (f1 and f2 where f2 > f1) produced simultaneously typically at two different levels (L1 and L2 where L1 > L2). When testing humans, the L1/L2 levels are typically 65/55 decibels (dB) sound pressure level (SPL; Marcrum et al., 2016). Fitzgerald and Prieve (2005) found that with primary levels (L2) ranging from 60 to 70 dB SPL, the mean amplitudes of the DPOAE responses were the highest. The two tones interact with each other and result in additional tones that are mathematical combinations (or distortion products) of both tones, one of which is 2f1-f2 (Marcrum et al., 2016). Humans with normal hearing generally have the most robust response at the 2f1-f2 distortion product (Christensen et al., 2015). The most robust distortion product is thought to occur when the excitation patterns produced by each primary tone have the greatest overlap (Marcrum et al., 2016). This overlap occurs between the f1 and f2 frequencies and is somewhat closer to f2. For that reason, the DP-gram is plotted with f2 on the horizontal axis. Distortion product otoacoustic emission tests examine OAEs in the frequency range of 0.8 to 8k Hz (Lonsbury-Martin et al., 1995). Otoacoustic emissions are evoked at or near frequencies where hearing thresholds are normal or near to normal (Kemp, 2002).

Extrinsic and intrinsic factors can affect OAE results. Extrinsic factors relate to influences that occur outside of the patient and intrinsic factors are variables within the patient. These factors can co-occur, or not, and impact the results from OAE testing.

Extrinsic Factors Affecting Distortion Product Otoacoustic Emissions

Monitoring cochlear function through DPOAEs means test consistency must be high and other factors that would negatively impact the reliability need to be taken into consideration when testing multiple times (Dreisbach et al., 2017). It is typical for there to be some degree of variability between tests, which is expected to be a difference in the DPOAE results of 5.3 or 6 to

8 dB between tests for the frequency ranges of 1 to 6k Hz or 6.5 to 7k Hz, respectively (Dreisbach et al., 2017). Dreisbach et al. (2017) and Kemp (2002) reported that positioning of the probe and probe fit had a huge impact on reliability and intensity of the OAE response, or lack thereof, between numerous tests. Re-seating the probe or altering placement between tests has an effect on variability as well since it could change background noise levels and affect how the resonances of the ear canal interact with the stimulus (Dreisbach et al., 2017). Background noise levels of greater than 40 dB could negatively impact OAE results (Kemp, 2002; Kemp et al., 1990). In terms of the types of background noise, continuous sounds and reverberant sounds are larger issues than sudden short sounds, which could be rejected by the equipment as the testing continues (Kemp, 2002). To help reduce background or ambient noise in the room, ensuring a good probe fit is imperative. An airtight seal reduces external noise and establishes correct positioning of the ports responsible for transmitting the signals to be fully facing the tympanic membrane and not blocked by the canal wall (Kemp et al., 1990).

Intrinsic Factors Affecting Distortion Product Otoacoustic Emissions

Intrinsic factors are factors that occur within the patient. The health status of the patient can be a factor in variability. The position of the patient could influence test-retest reliability since changing positions between tests could impact DPOAE magnitudes and cause variation between tests (Voss et al., 2006). Voss et al. (2006) reported DPOAEs decreased in magnitude as posture went from upright to horizontal. Excessive cerumen, fluid in the middle ear, and infections are other factors that could prevent successful and accurate OAE recordings (Kemp, 2002). Hearing sensitivity status could also impact OAE testing as a conductive hearing loss could prevent OAEs from being present or measurable. Significant sensorineural hearing loss could result in lack of or reduction in DPOAE responses.

Some other intrinsic factors that could impact DPOAE responses result from the vulnerability of the outer hair cells in the inner ear of the auditory system. Because of the vulnerability of outer hair cells to anoxia, ototoxic substances, and noise, if damage occurs, then it could impact DPOAE responses. As soon as the ear loses access to oxygen, it becomes damaged because it causes the auditory system to have blood supply loss. The outer hair cells are the first structures to become damaged and/or lost with loss of oxygen. Outer hair cells are especially vulnerable to ototoxic substances and could become damaged and/or lost when ototoxic substances are introduced into the body. Exposure to excessive sound levels could cause damage and/or loss of outer hair cells, which could result in a reduction of OAE responses (Hall, 2000; Robinette & Glattke, 2007).

F_1 and F_2 Distortion Product Otoacoustic Emission Stimulus Tones

Because OAEs are frequency-specific responses, the measured frequency from an OAE is significant (Kemp, 2002). The primary location the 2f1-f2 generates on the basilar membrane is in the region of the f2 frequency (Zelle et al., 2015); then the generation travels to the basal end of the cochlea and out through the middle ear to the ear canal where it is measured. It was found that when the f1 and f2 levels were equal in dB SPL, the location of f2's peak was greater in intensity than f1 (Kemp, 2002). Therefore, by making f1 a greater intensity, the peak would shift to the location of f2's peak (Kemp, 2002; Zelle et al., 2015). The peak of f2 changes according to different frequencies and there is a complex interaction between f1 and f2 levels that varies with changes in frequency (Zelle et al., 2015). This would increase the production of distortion products. For humans, there is an ideal stimulus intensity ratio (f2: f1) to get the maximum production of distortion products (Kemp, 2002). "The extent to which all the elemental OHC (outer hair cell) DP (distortion product) sources reinforce each other to produce a strong backwards DP travelling wave (and hence a DPOAE) depends on the spatial distribution of DP phases” (Kemp, 2002, p. 234). The velocities of the traveling waves of f1 and f2 are what determines if the distortion product produces or does not produce a DPOAE response.

When the velocity ratio of f1 and f2 is one, or close to one (i.e., 1.02), the traveling wave velocities are very similar, resulting in a wave that travels toward the apical end of the cochlea. This means not much of a traveling wave gets sent back to create a DPOAE. With a large f2/f1 ratio (i.e., 1.5) the distortion product phase distribution would mostly cancel itself out, causing very small spreading of the distortion product wave. It was found that there is an optimum f2/f1 ratio for humans, around 1.2, that causes distortion product distribution to create a traveling wave moving backward over the outer hair cells (Kemp, 2002). Harris et al. (1989) found that at a low ratio of around 1.01, DPOAEs decreased until they reached a minimum amplitude and that as the ratio increased in the 1.18 to 1.28 range, the DPOAEs increased until they reached a maximum amplitude. If the ratio increased past the one that elicited the maximum peak amplitude response (range of 1.4), the DPOAE again decreased until it reached a minimum amplitude. Currently, for humans, the ratio of f2/f1 is typically 1.2 to 1.25 for clinical use (Abdala, 1996; Abdala & Visser-Dumont, 2001; Knight & Kemp, 2000). With the ratio around 1.2-1.25 between the two stimulus tones, it allows for the forward traveling waves to produce a backward traveling wave with the greatest amplitude to then be measured by the OAE equipment. It was found this ratio allowed for OAE test results on humans to have the greatest amplitudes and best recordings.

Additionally, it is known that mammalian ears, including human ears, do not only produce the 2f1-f2 distortion product. Multiple distortion products are produced; however, it has been established that the 2f1-f2 distortion product has the largest amplitude for human ears.

Therefore, the 2f1-f2 distortion product is the distortion product that is recorded in human ears as standard practice. It is possible that due to differences in ear anatomy and physiology, canine ears might respond differently than human ears to probe frequencies f1 and f2. As a result, canine ears might produce distortion products at different mathematical combinations than human ears. It is theorized the distortion product mathematical combination might be larger in canine ears because of the size differences in ear anatomy compared to humans. Further study is necessary to determine if canine ears produce distortion products at levels that vary compared to humans.

Test Protocol

Otoacoustic Emission Equipment

The probe is designed to bring the transducer within millimeters of the probe tip to get good responses and frequency bandwidths for a variety of ears (Kemp et al., 1990). The probe tip is a rubber tip or a disposable foam tip (Knight & Kemp, 2000) that fits on the end of the probe. It is set into the ear canal to the point where the rubber flange on the tip is fully compressed to create a seal (Kemp et al., 1990). The probe contains ports that produce the stimuli and receive the signals transmitted back into the canal. The probe needs to be placed deeply in the ear canal for maximum OAE responses and maximum noise levels are reduced (Kemp, 2002). Distortion product otoacoustic emission stimuli consist of two pure tones; typically, the lower frequency tone is produced at 60-70 dB SPL and the higher frequency tone is produced at a lower level of 50-70 dB SPL (Kemp, 2002). Since OAEs are very low-level sounds, any external sounds above 40-45 dB SPL could trigger the data to be rejected (Kemp et al., 1990) so an environment with low levels of ambient noise is necessary to perform OAE testing. Noise that is lower in level (not rejected) could still be discriminated against because the OAE software system could determine what is noise through the signal components in a process called signal averaging. For example, low level noise is unrelated in phase and frequency content in comparison to the stimuli and when the signals are averaged together, it will cancel out the noise (Kemp et al., 1990). Even though the system could discriminate noise from stimuli and emissions through signal averaging, tests still need to be run multiple times to confirm the noise has in fact been eliminated appropriately (Kemp et al., 1990).

Distortion product otoacoustic emissions are recorded on what is called a DP-gram. The DP-gram is a display of the amplitude as a function of the frequency of f2 (Abdala & Visser-Dumont, 2001). The frequency plotted on the DP-gram is generally the frequency of the f2 primary tone, not the frequency of the DPOAE, because past research found the interaction of the primary tones occurred where the traveling waves of the primary tones overlapped the most (Robinette & Glattke, 2007). Where the primary tones overlap the most is between the f1 and f2 frequencies, with the tendency to be closer to f2, which is why the DP-gram is plotted with f2 on the horizontal axis. The DP-gram records information of the OAE as well as the level of background noise—called the noise floor. A DPOAE is present (considered a pass) if the level of the DPOAE plus the noise floor is greater by a criterion amount (e.g., 6 dB) than the average noise level (Robinette & Glattke, 2007). A DPOAE test is considered absent or present but abnormal when a response is not present for at least two f2 frequencies (Abdala & Visser-Dumont, 2001).

Mutt Muffs

Mutt Muffs® (Safe & Sound Pets, 2021) might help reduce ambient noise in the environment that could prevent OAEs from being accurate and repeatable. Mutt Muffs provide passive hearing protection for canines (Safe & Sound Pets, 2021). The hearing protection consists of circumaural muffs that are connected by a strap that could be adjusted based on the canine's head size. Mutt Muffs come in different sizes to accommodate different breeds and sizes of dogs (Safe & Sound Pets, 2021). According to the manufacturers of Mutt Muffs, the inner foam on the muffs is the same foam that is used in high end commercial headsets for humans. They estimate the reduction level is approximately 25 to 28 dB with the manufacturers assuming there is a good seal of the muffs against the canine's head and a good fit completely around the ears (Safe & Sound Pets, 2021). The manufacturers did state the only data they had was through 20,000 Hz, which is not the complete range for canine hearing. This covers the range for humans; however, canines can hear from approximately 67 Hz to 45k Hz (Strain, 2012). Since there are no data on the higher frequencies, the manufacturers could not be sure of the decibel reduction at those higher frequencies; however, they said the effectiveness of the Mutt Muffs increased as the frequency of the stimulus increased since higher frequencies were more easily absorbed (Safe & Sound Pets, 2021). The Mutt Muffs manufacturers claimed that canines with pendulous ears had another layer of protection since the pinnae were pressed down by the Mutt Muffs, which covered the ear canal; however, no data confirmed this.

Overview of Otoacoustic Emissions in Canines

Otoacoustic emissions testing could provide important and useful information regarding the integrity of the canine's auditory system (Schemera, 2011). Two types of emissions testing are used for diagnostics and screenings: transient evoked otoacoustic emissions (TEOAE) testing and DPOAE testing. Transient evoked otoacoustic emissions testing uses transient stimuli (e.g., clicks) and DPOAE testing uses pairs of tones (Schemera, 2011). It is currently not common for DPOAE or TEOAE assessments to be used in private veterinary offices (Schemera, 2011). There are very few records of studies on evoked OAEs in canines. Studies that have been done include a study of DPOAEs in clinically normal alert puppies (Schemera, 2011), preliminary findings of TEOAEs and DPOAEs in canines (Sockalingam et al., 1998), TEOAEs and DPOAEs in canines to assess cochlear hearing status (Goncalves et al., 2012), DPOAEs in geriatric dogs (Strain et al., 2016), and deafness detection in puppies using an OAE screener (Sims et al., 2017). Table 1 provides a summary for each of the aforementioned studies followed by a more detailed discussion of the research.

Table 1

Summary Table of Canine Distortion Product Otoacoustic Emissions Research

Author(s), (Year)	Sample Size	Test Used	Test Settings	General Findings
Goncalves et al. (2012)	53	DPOAE TEOAE BAER	• Noise-reducing ear cover over canine's ears • TEOAE stimulus level: 90 dB SPL • DPOAE stimulus level: 75/55 dB SPL • F2/f1 ratio: 1.21	• Two groups: first had DPOAEs and TEOAEs compared, second had OAEs and BAER compared • 19 breeds, 3 months-10 years old, 5-50 kg • TEOAEs and DPOAEs successful in determining hearing status in canines • Able to get accurate TEOAE and DPOAE results and accurately identified pass for ears where BAER responses present, and refer for ears where BAER responses were not present
Schemera (2011)	23	DPOAE	• Acceptance criterion: result 8 dB or greater above noise floor • F2/f1 ratio: 1.20 • Stimulus levels: 65/55 dB SPL • 13 mm foam probe tip	• 7.5-10.5-week-old puppies with normal hearing sensitivity • DPOAEs were robust, repeatable, and reliable • DPOAEs in agreement with BAER behavioral testing results • Mean DPOAE amplitudes not significantly different between ears • Mean DPOAE amplitudes not significantly different between genders • Amplitudes of DPOAEs increased significantly as f2 increased, in both left and right ears • Concluded that DPOAE absolute amplitudes of puppies were comparable to previously recorded of adult canines (anesthetized)
Sims et al. (2017)	34	DPOAE BAER	• 13 mm foam probe tip • Stimulus levels: 65/55 dB SPL	• Puppies 6-10 weeks old • All ears with normal BAER test results had DPOAE results that were a pass • Ears with flat BAER test results had DPOAE results that were a refer • Concluded DPOAE screening can reveal pass/refer results in agreement with BAER test result •

Table 1 Continued

Author(s), (Year)	Sample Size	Test Used	Test Settings	General Findings
Sockalingam et al. (1998)	7	DPOAE TEOAE	F2/f1 ratio: 1.22	• Adult canines with normal hearing sensitivity • Able to use human test specifications to get TEOAE results • Adjustments to SNR for DPOAEs were necessary to get results • Stimulus levels to produce DPOAEs found to be 55/55, 55/45, and 55/35 • TEOAEs and DPOAEs recorded twice without removing probe for repeatability • Reproducibility of TEOAEs had variability • No consistent configuration of frequency spectrum between dogs for TEOAEs
Strain et al. (2016)	28	DPOAE BAER	• 9 mm or 10 mm probe tip • Stimulus levels: 65/55 dB SPL • F2/f1 ratio: 1.21	• Geriatric canines (mean age 12.2 ± 2.2 years) • Control group of adult canines (5.9 ± 3.0 years) • Compared BAER responses to DPOAE responses • Found significant decreases in DPOAE response amplitudes for 6-12k Hz in geriatric canines (compared to control group) • BAER responses similar to DPOAE responses: both decreased for geriatric population • Concluded DPOAE testing on canines could be used to track hearing changes with age in canines

Sockalingam et al. (1998) reported successful recording of DPOAEs and TEOAEs in the ears of seven canines with normal hearing sensitivity. The purpose of the study was to get preliminary results on TEOAEs and DPOAEs in dogs and determine some basic characteristics. The researchers used the default settings for testing TEOAEs in humans and were able to get results in 92% of canine ears tested (Sockalingam et al., 1998). When Sockalingam et al. tested TEOAEs using the quick screen tool, they were able to get results in 100% of the canine ears. The researchers reported they were able to use human test specifications to get results for TEOAEs but adjustments of the signal-to-noise ratio (SNR) had to be made for measuring DPOAES in canines. The researchers found the stimulus levels to produce DPOAEs were 55/55, 55/45, and 55/35 dB SPL after looking at eight different stimulus levels (L1/L2: 70/70, 65/65, 65/55, 60/60, 55/55, 65/45, 55/45, 55/35 dB SPL; Sockalingam et al., 1998). The f2/f1 ratio was set at 1.22 with the researchers reasoning the ratio of 1.22 provided the most robust DPOAEs in humans and some laboratory animals. For all the testing, the researchers placed a B type probe designed for adults in the canine ear canal. Both TEOAEs and DPOAEs were recorded twice without moving the probe to confirm repeatability. The reproducibility for TEOAEs had a large range of variability but the average percentage was above 60%, which was the criterion at the time of the study to determine if TEOAEs were present in humans. Sockalingam et al. concluded there was no consistent configuration of frequency spectrum between the dogs for TEOAEs. Size, age, breed, and ear canal size were not controlled for this study and Sockalingam et al. recommended that further study be performed to provide more detailed information about TEOAEs and DPOAEs in canines.

In 2011, Schemera evaluated DPOAEs in 23 puppies with normal hearing aged 7.5-10.5 weeks old. Distortion product otoacoustic emissions were recorded on all the puppies and were repeated one to two times for repeatability measures. The criterion for acceptance of the result was required to be 8 dB or greater above the noise floor. The DPOAEs were robust, repeatable, reliable, and in agreement with auditory brainstem (BAER testing) and behavioral testing results. Stimulus levels of 65 (f1) and 55 (f2) dB SPL and an f2/f1 ratio of 1.2 were used for the study. The probe tip consisted of a 13mm foam tip that was trimmed to allow the tubing port in the center of the foam tip to sit flush with the probe assembly port, all of which were attached to the probe unit. The foam tip was placed at the entrance to the horizontal portion of the puppies' ear canals. The results from the study were as follows: mean DPOAE amplitudes were not significantly different between ears (left and right) and mean DPOAE amplitudes were not significantly different between genders (male and female). They did find the amplitudes of the DPOAEs increased significantly as f2 increased in both the left and right ears of the canines. Schemera concluded the DPOAE absolute amplitudes of the puppies were comparable to those previously recorded of adult canines that had been anesthetized. The results were indicative that DPOAE testing could be performed on puppies and that accurate, repeatable, and reliable results could be obtained.

In 2012, Goncalves et al. performed a study evaluating hearing status in canines through the use of TEOAEs and DPOAEs. The researchers performed testing on two groups of anesthetized dogs: the first group had their OAE results compared to BAER test results and the second group had their TEOAE responses and DPOAE responses compared. The 53 canines were a combination of males and females, consisted of 19 breeds, ages were 3 months to 10 years, and ranged in weight from 5 to 50 kg. They found TEOAE and DPOAE testing was successful in determining hearing status in canines (deaf or hearing). The researchers used probe tips according to ear canal size of the canine. In addition to the probe tip, the researchers placed a noise-reducing ear cover from Parkson Safety Industrial Corporation loosely over the canine's ear (manufacturer stated noise reduction of 31-37.9 dB). The stimulus level for TEOAEs was 90 dB SPL. For DPOAEs, the stimulus levels were 75 dB SPL for f1 and 55 dB SPL for f2, and the frequency ratio of f2/f1 was 1.21. Goncalves et al. concluded that through using similar criteria for pass/fail of OAEs, they were able to get accurate TEOAE and DPOAE results and accurately identified a pass for ears where BAER responses were present and a refer for ears where BAER responses were not present. The researchers reported that further research needed to be performed to determine optimal pass/refer criteria for canines.

In contrast to Schemera (2011), Strain et al. (2016) evaluated DPOAEs in 28 geriatric canines. Strain et al. conducted DPOAE testing on geriatric canines (mean age of 12.2 with a standard deviation of ± 2.2 years). A control group of adult canines (5.9 ± 3.0 years) was also tested for comparison of frequency-specific changes in the cochlear responses seen through DPOAEs. They used a 9mm or 10mm probe tip for the DPOAE testing and the stimulus levels were 65 dB SPL and 55 dB SPL for f1 and f2. The f2/f1 ratio was 1.21. The frequency range tested was six primary frequencies at 2k Hz intervals from 2-12k Hz. A minimum of two DPOAE tests were run for each ear to confirm repeatability. In addition, the researchers measured BAER responses to compare to the DPOAE results. Strain et al. found significant decreases in DPOAE response amplitudes in the frequency range of 6-12k Hz in the geriatric canines when compared to the control group of adult canines. The researchers reported the BAER responses were similar to the DPOAE responses as both decreased for the geriatric population. When the DPOAE results had small amplitudes, the BAER testing results corresponded with reduced amplitude as well. The geriatric canine population had a significant reduction in the response of the outer hair cells for the frequency region of 6-12k Hz, which coincided with aging changes that become present in the geriatric population of canines. Strain et al. concluded that DPOAE testing on canines could be used to track hearing changes with age in canines.

In 2017, Sims et al. tested 34 puppies using DPOAEs with the purpose of deafness detection. The puppies were aged 6-10 weeks old, both male and female from a variety of breeds, and the DPOAE responses were compared to BAER test results. Sims et al. found that in all the ears with normal BAER test results, the DPOAE results were a pass. Puppies that had flat BAER test results had DPOAE results that were a refer. Sims et al. used a 13mm foam probe tip and a handheld DPOAE screener to perform the testing on the puppies. The stimulus levels were 65 dB SPL and 55 dB SPL for f1 and f2, respectively. Sims et al. concluded that although the Orthopedic Foundation for Animals reported BAER testing was the only acceptable hearing assessment method for canines, DPOAE screening could reveal pass/refer results that were in agreement with BAER test results and were not affected by age, sex, breed, state of consciousness, or ear canal size.

Scheifele and Clark (2012) noted in their description of electrodiagnostic evaluation of auditory function in canines that one of the issues regarding performing OAEs on canines was it was difficult to get a good seal of the probe tip. Lack of a good seal meant poor SNRs, discomfort for the canine, and inability to get accurate and repeatable results. Scheifele and Clark concluded that although BAER testing has been the method used to determine hearing status in canines in veterinary offices, with better data, protocol, and equipment, OAEs could be of use to veterinary clinics to perform hearing screenings on canines as well as diagnostic evaluation in combination with the BAER test.

Current research delved into OAEs in canines; however, still considerable information was missing. For diagnostic and screening purposes, it is imperative that norms and guidelines be determined. This would allow clinicians to get accurate test results when conducting OAEs on canines, which would result in more accurate diagnoses of canines' hearing sensitivity. Specifically, DPOAEs rely on specific f1 and f2 ratios, signal levels, probe fit, environmental specifications, and patient cooperation. Complex interactions exist among all these variables and there are still unanswered questions regarding exactly where along the basilar membrane the DPOAE is generated in human ears. As aforementioned, the canine cochlea contains more turns than humans, which means their basilar membrane is likely longer. This could be the reason canines have a larger range of frequencies audible to them. With this information, it could be inferred that the optimal f2/f1 ratio might be different than it is for humans since for humans, it is specifically related to the basilar membrane length, basilar membrane distance between frequencies, and how the f1 and f2 frequencies create traveling waves. The aim of this study was to determine what stimulus frequency ratios produced the most robust DPOAEs in healthy canine ears.

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CHAPTER III

METHODOLOGY

Subjects

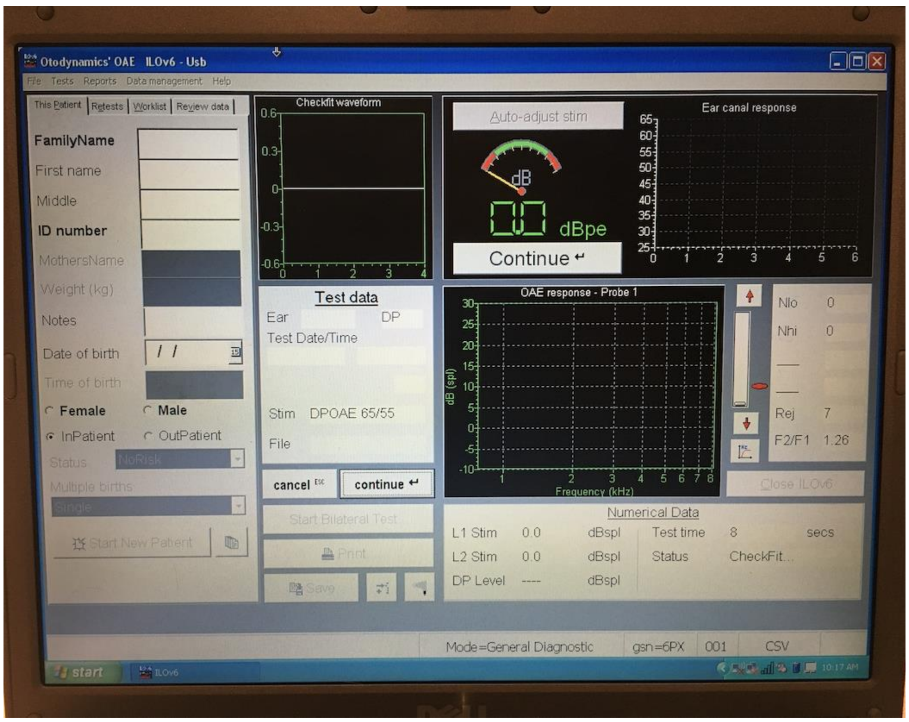
Ten dogs with normal hearing were recruited for DPOAE testing. Each canine was tested once (during one session). The owners voluntarily brought the subjects to the University of Northern Colorado Speech-Language Pathology and Audiology Clinic. The canines chosen for the study were a convenience sample recruited through The Facility for Education and Testing of Canine Hearing and Laboratory for Animal Bioacoustics (FETCHLAB) at the University of Northern Colorado. No attempt was made to control for breed or gender. Inclusion criteria were the canines must be between the age of two and eight years old, weigh 10-90 lbs. due to limitation of the size of available Mutt Muffs, and have essentially normal hearing sensitivity confirmed through robust and repeatable DPOAE responses during testing. Case history was acquired for each canine to confirm there were no current conditions that could have negatively impacted testing (i.e., cerumen impaction, otitis media, etc.). Performance of DPOAE testing of canines at FETCHLAB at the University of Northern Colorado was approved by the Institutional Animal Care and Use Committee, which is responsible for determining research is ethically and humanely conducted (see Appendix A).

Test Environment, Procedure, and Instrumentation

Testing was performed using the Otodynamics Ltd. ILO-v6 clinical OAE software (serial# DP4/04/0132/11) with up-to-date calibration (calibrated within 12 months of testing). Figure 1 provides an image of the software.

Figure 1

Image of the Otodynamics Ltd. ILO-v6 Software



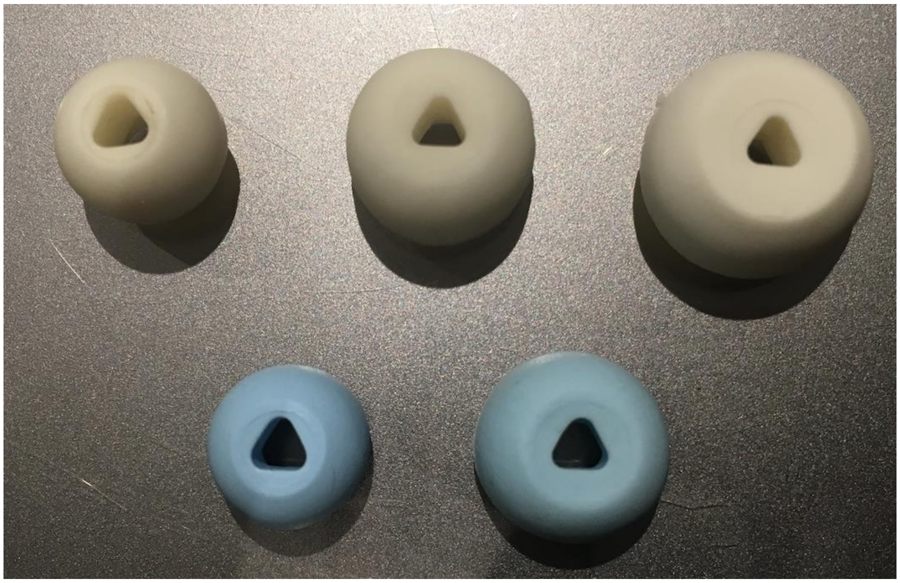
Mutt Muffs (Safe & Sound Pets, 2021) were utilized to help reduce ambient noise levels because the probe tips were not designed specifically to fit the canine ear. This could result in lack of the probe tip creating a complete seal; therefore, ambient noise would potentially be a larger factor for canines. The appropriate size of Mutt Muffs used during testing was determined by the ability to cover the entire canine ear and be secured with Velcro around the head. Mutt

Muffs were determined to be necessary for testing purposes to reduce ambient noise in the testing environment. The ILO-v6 software was set up to perform a diagnostic DPOAE test. Distortion product otoacoustic emissions testing was performed on the right ear only for each canine. The testing environment was a single-walled sound-treated booth, Tracoustics acoustical enclosure (serial# 477-076068), in the University of Northern Colorado Speech-Language Pathology and Audiology Clinic.

Distortion product otoacoustic emissions were performed on all 10 canines without the use of sedation or anesthesia. Otoscopy was utilized to confirm the ear canal was clear and to aid in selection of an appropriately sized rubber probe tip from Otodynamics Ltd. The OAE probe tips of various sizes (Type R9M, R11M, R13M) came in blue and white and specifically designed for use with the ILO-v6 clinical OAE software system (see Figure 2).

Figure 2

Otodynamics Ltd. Probe Tips of Various Sizes to be Used in Distortion Product Otoacoustic Emissions Testing



The probe tip was attached to the probe and subsequently inserted into the vertical portion of the ear canal to the ridge where it turned into the horizontal canal. The adequately sized Mutt Muffs (Safe & Sound Pets, 2021) were placed and secured with Velcro to cover the ear being tested. Two attempts to conduct DPOAEs using an optimal f2/f1 ratio of 1.22 for humans (Abdala & Visser-Dumont, 2001; Knight & Kemp, 2000) were completed using the ILO-v6 software system to confirm reliability of the initial test results. The settings were kept the same for each test on every canine. The L2 was fixed at 55 dB SPL and the L1 was fixed at 65 dB SPL. The f1 was varied to produce nine total f2/f1 frequency ratios while f2 remained fixed. A calibration test was run prior to testing each canine. For each test, there was an initial check-fit test. To pass the check-fit test, there needed to be at least 30 good check-fit sweeps. When the test was being performed, the minimal criterion was two DP loops with a minimum response level in dB of -10. The test automatically timed out at three minutes. The noise reject level was set to 10 dB SPL[1]. The stopping criteria for the testing was two completed DP loops. If the canine moved or vocalized during the testing but did not affect the responses or noise floor, the testing continued. If canine movement or vocalizations resulted in the noise floor going above 10 dB SPL[1] for two to three frequencies, then a third DP loop was performed. If the canine continued to have movements or vocalizations that caused more than three frequencies to have noise floors above 10 dB SPL, then the testing was stopped and restarted once the canine was cooperative. When two repeatable responses were initially obtained, the f1 was then altered to produce the following f2/f1 ratios: 1.18, 1.20, 1.21, 1.22, 1.23, 1.24, 1.25, 1.26, and 1.28. There was one run of the diagnostic DPOAE test for each ratio and the test was only run a second time

[1] Originally 5 dB SPL, however this was quickly found to be unrealistic during data collection. Therefore, it was adjusted to 10 dB SPL.

if the response was impacted by the canine's movement or vocalizations and an accurate response needed to be determined through repeatability. The order of the frequency ratios was randomized across the subjects. It was important to mention that once the probe tip was inserted and the Mutt Muffs were secure, the probe tip was not removed and reinserted. The DP chart type was a DP line graph with four points per octave from 1-8 kHz. The data were a DP-gram and the data recorded consisted of the absolute amplitude of the DPOAE and the SNR. The data were saved on the computer as well as copied onto a USB drive.

Distortion product otoacoustic emissions results were collected once the probe was placed into the vertical canal portion of the canine's ear canal and Mutt Muffs (Safe & Sound Pets, 2021) were placed securely around the head. Repositioning of the probe and Mutt Muffs was common and occurred when the subject shook his/her head in an effort to remove the equipment. There were numerous incomplete test runs for all of the test subjects, especially at the beginning of testing due to canine noise or movement that happened before the canine settled down. Incomplete test runs were not documented and were discarded at the time of data collection. Complete runs were obtained once the canine and the environment were conducive to accurate data collection. The DPOAE line graph representations for each f2/f1 ratio for all test subjects are in Appendix B.

Descriptive and nonparametric statistics (Kruskal-Wallis test) were used to determine the f2/f1 ratio that elicited the most robust responses for canine ears.

CHAPTER IV

RESULTS

Successful results were obtained for 10 out of 10 subjects for all 9 f2/f1 ratios. Table 2 provides descriptions of subject demographics. Figure 3 illustrates the results from subject 4 for all f2/f1 ratios tested.

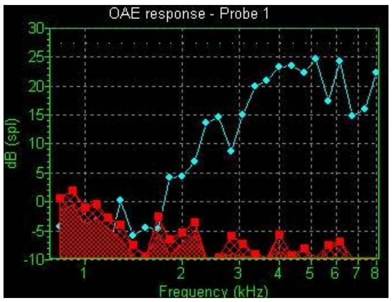
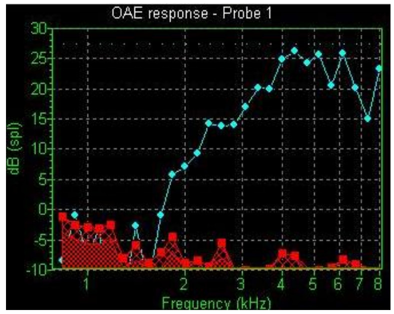
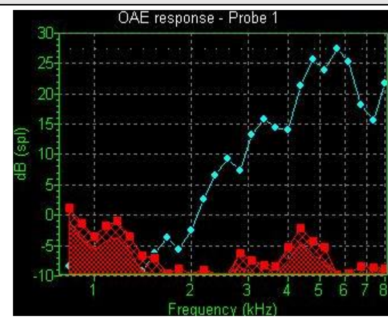
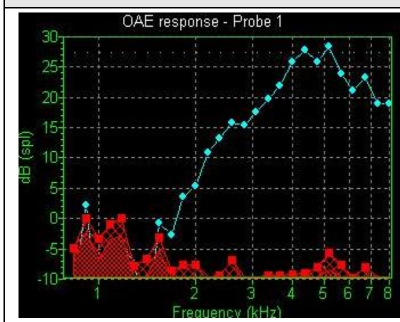
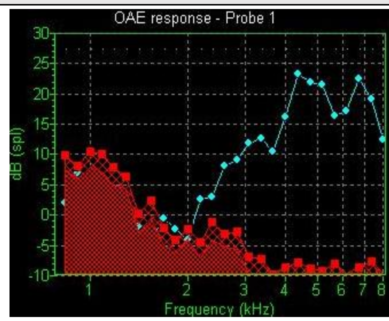
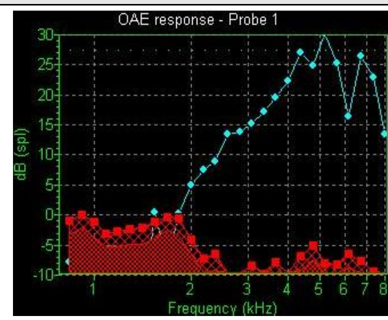
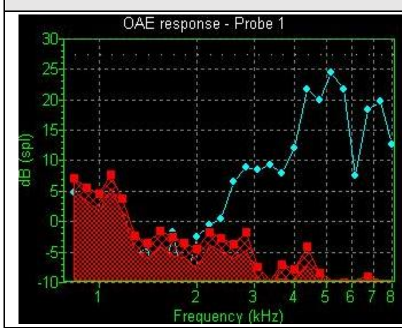
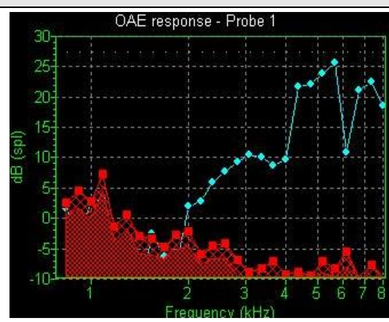
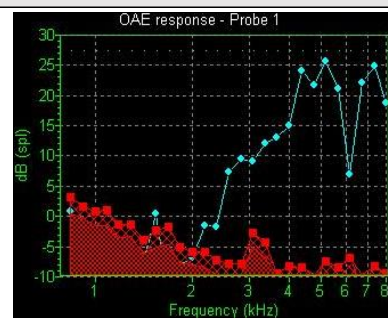
Table 2

Descriptions of Test Subjects

Subject	Breed	Approximate Age (yrs.)	General Size (lbs.)	Gender
1	Mix	7	Small (10-30)	M
2	Border Collie	4	Medium (30-55)	M
3	German Shepherd	3	Large (55+)	M
4	Mix	2	Medium (30-55)	M
5	Australian Shepherd	4	Medium (30-55)	F
6	Labrador Retriever	6	Large (55+)	M
7	Labrador Retriever	8	Large (55+)	M
8	Labrador Retriever	4	Large (55+)	M
9	Labrador Retriever	8	Large (55+)	F
10	Mix	4	Small (10-30)	F

Figure 3

Distortion Product Otoacoustic Emissions Response Line Graphs for Subject 4 for all Nine f2/f1 Ratios Tested

1.18	**1.20**	**1.21**
		
1.22	**1.23**	**1.24**
		
1.25	**1.26**	**1.28**
		

The mean DPOAE amplitudes are displayed in Figure 4 and Table 3. Appendix C provides the mean data obtained for each subject individually.

Figure 4

Average Distortion Product Otoacoustic Emissions Amplitude Values at Each f2 Frequency Tested for Each f2/f1 Ratio

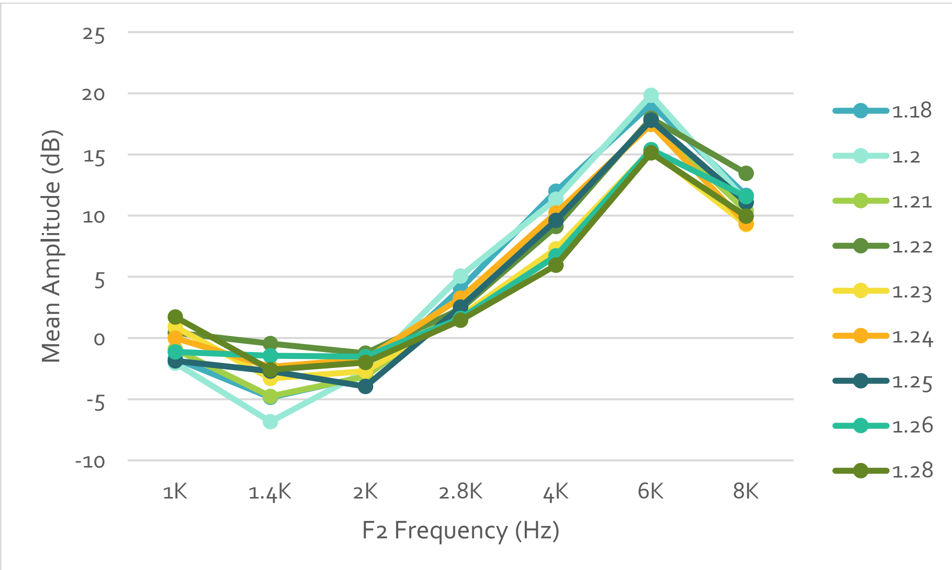


Table 3

Mean Amplitudes in Decibel Sound Pressure Level for Each Frequency Tested

	1k Hz	1.4k Hz	2k Hz	2.8k Hz	4k Hz	6k Hz	8k Hz
M	-0.513	-3.26	-2.456	2.741	9.09	17.303	10.881
Minimum	-2.06	-6.84	-3.96	1.46	5.94	15.12	9.28
Maximum	1.72	-0.46	-1.52	5.05	11.34	19.85	13.45
SD	1.336	1.945	0.895	1.188	2.064	1.689	1.315

N = 9

Figures 5-8 illustrate the amplitudes in dB for all f/2/f1 ratios for each subject at the individual frequencies of 2.8k Hz, 4k Hz, 6k Hz and 8k Hz. From the figures, it can be noted that subjects 2, 3, and 6 had overall reduced amplitudes when compared to the other subjects. It also showed no significantly different amplitudes for different f2/f1 ratios.

Figure 5

Amplitudes at 2.8k Hz for All f2/f1 Ratios for Each Subject

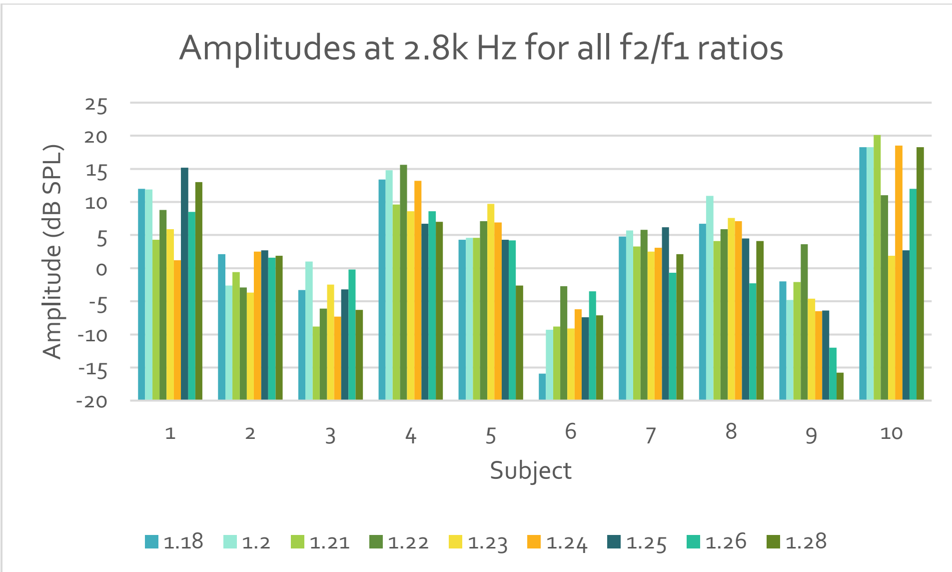


Figure 6

Amplitudes at 4k Hz for All f2/f1 Ratios for Each Subject

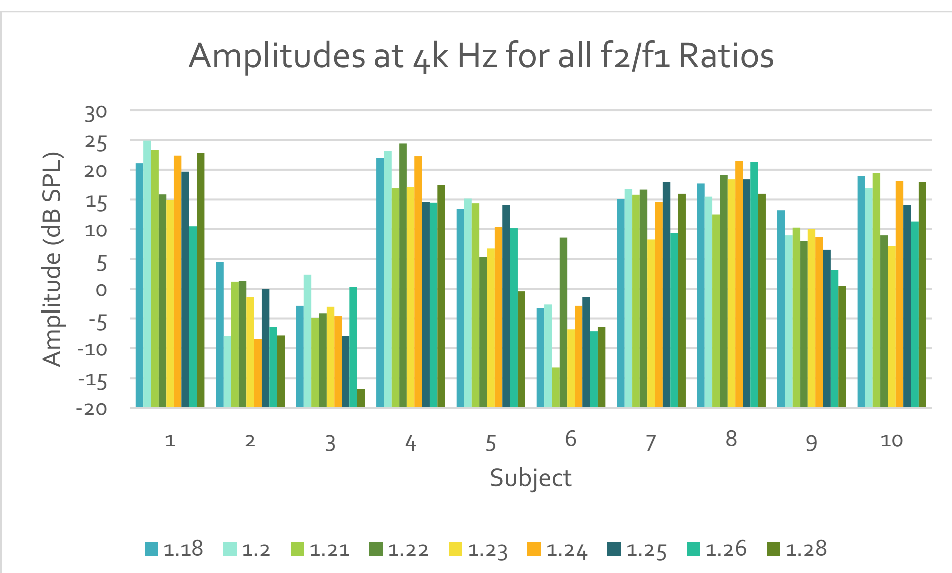


Figure 7

Amplitudes at 4k Hz for All f2/f1 Ratios for Each Subject

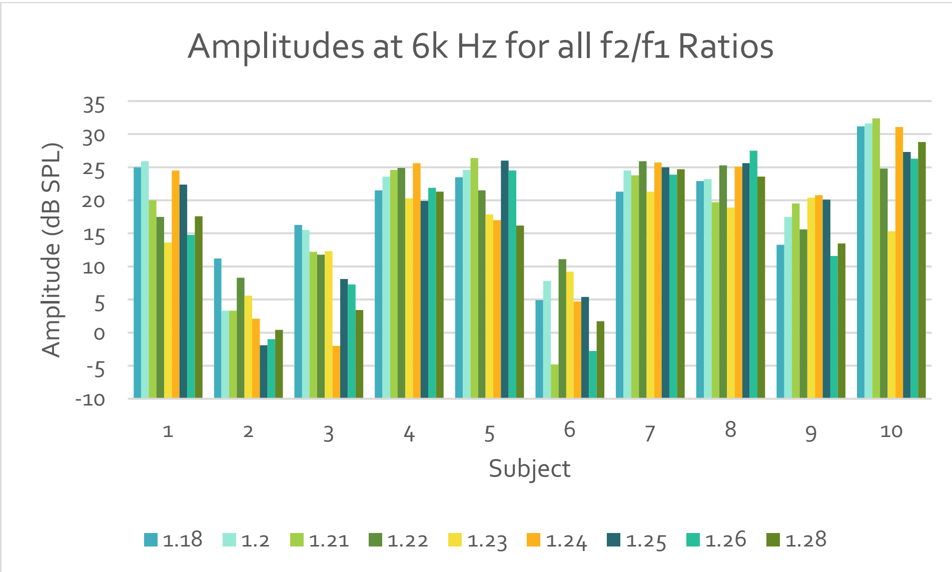
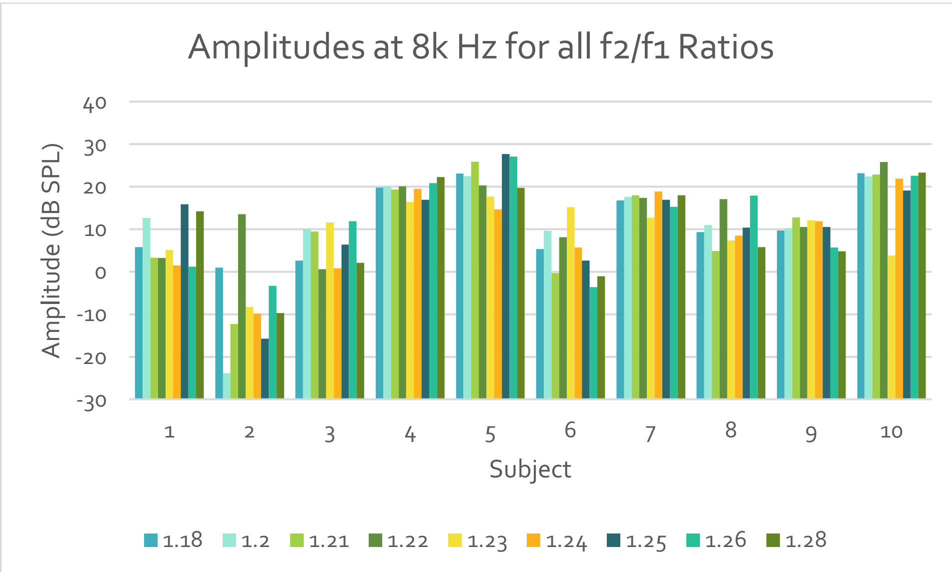


Figure 8

Amplitudes at 8k Hz for All f2/f1 Ratios for Each Subject



An analysis of variance was run to evaluate the statistical significance of amplitude across frequencies for the f2/f1 ratios tested (see Table 4).

Table 4

Amplitude Results from the Repeated Measures Analysis of Variance Using Pillai's Trace for Ratio and Frequency

Effect	Value	*F*	Hypothesis df	Error df	Sig
Ratio	0.850	1.413	8.000	2.000	0.479
Frequency	0.979	31.157	6.000	4.000	0.003

Results showed no statistically significant differences in DPOAE amplitudes across ratios. The version of SPSS used was IBM SPSS Statistics, Version 25. Repeated measures analysis of variance (ANOVA) revealed a *p*-value of .479 ($p < .05$). On average, 6k Hz had the largest amplitude with a mean of 17.303 dB, followed by 8k Hz with a mean amplitude of 10.881 dB. At and below the frequency 2k Hz, the mean amplitudes were less than 0 dB. These results are depicted in Figure 8.

In addition to amplitudes, noise levels were also analyzed. Noise level trends are illustrated in Figure 9 and Tables 5 and 6.

Figure 9

Average Noise Values in Decibel Sound Pressure Level Across All Subjects at Each Frequency Tested for Each f2/f1 Ratio

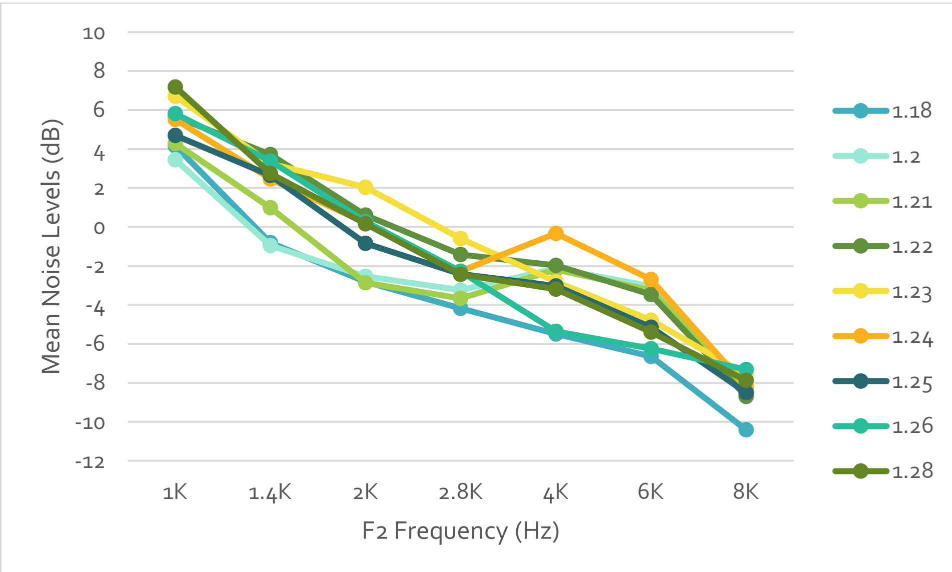


Table 5

Noise Level for Each Frequency Tested

	1k Hz	1.4k Hz	2k Hz	2.8k Hz	4k Hz	6k Hz	8k Hz
M	5.277	1.937	-0.636	-2.498	-2.944	-4.523	-8.336
Minimum	3.45	-0.95	-2.86	-4.18	-5.49	-6.65	-10.41
Maximum	7.19	3.71	2.04	-0.59	-0.33	-2.7	-7.32
RNG	3.74	4.66	4.9	3.59	5.16	3.95	3.09

N = 9

An analysis of variance was run to evaluate the statistical significance of noise floor levels across frequencies for the f2/f1 ratios tested (see Table 6).

Table 6

Noise Floor Results from the Repeated Measures Analysis of Variance Using Pillai's Trace for Ratio and Frequency

Effect	Value	*F*	Hypothesis df	Error df	Sig
Ratio	0.906	2.408	8.000	2.000	0.326
Frequency	0.986	47.555	6.000	4.000	0.001

Results showed no statistically significant differences in DPOAE noise floors across ratios. The version of SPSS used was IBM SPSS Statistics, Version 25. Repeated measures ANOVA revealed a *p*-value of .326 ($p < .05$). On average, 8k Hz had the lowest noise level with a mean of -8.336 dB, followed by 6k Hz with a mean noise level of -4.523 dB. At and below the frequency 1.4k Hz, the mean noise levels were above 1 dB. These results were depicted in Figure 9.

In addition to amplitude (in dB SPL), DPOAE results are reported clinically for signal-to noise-ratios, that is, how much larger than the noise floor the DPOAE response is. Signal-to-noise ratio trends are illustrated in Figures 10, 11, and 12.

Figure 10

Average Distortion Product Otoacoustic Emissions Signal-to-Noise Ratios (in Decibels) Across All Subjects at Each Frequency Tested Averaged Across All f2/f1 Ratios

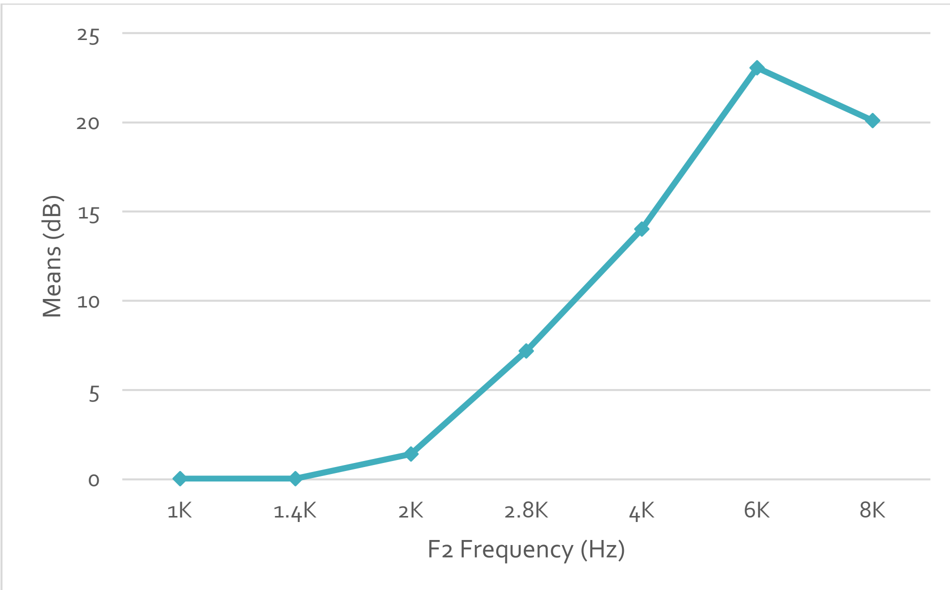


Figure 11

Average Signal-to-Noise Ratio Values (in Decibels) Across All Subjects at Each Frequency Tested for Each f2/f1 Ratio

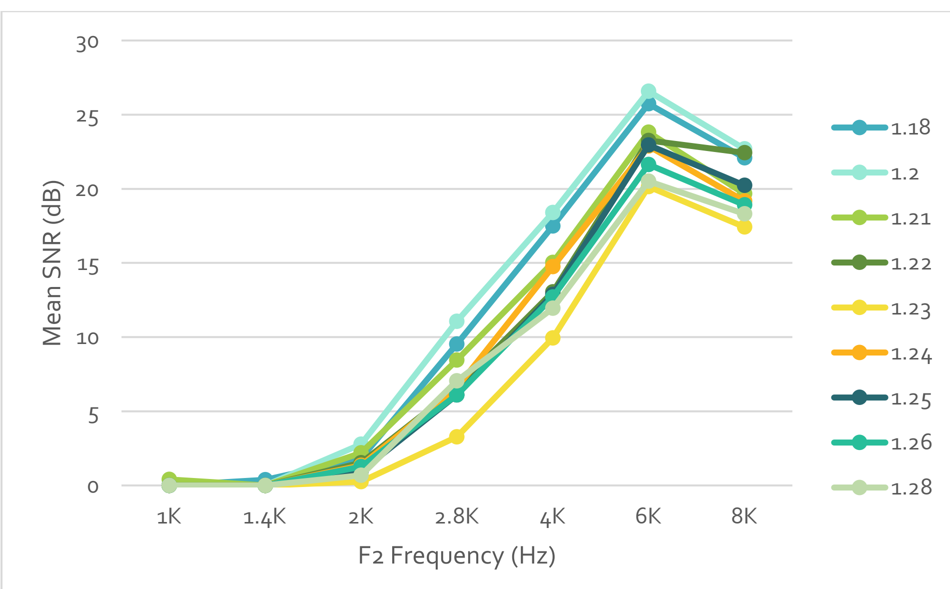
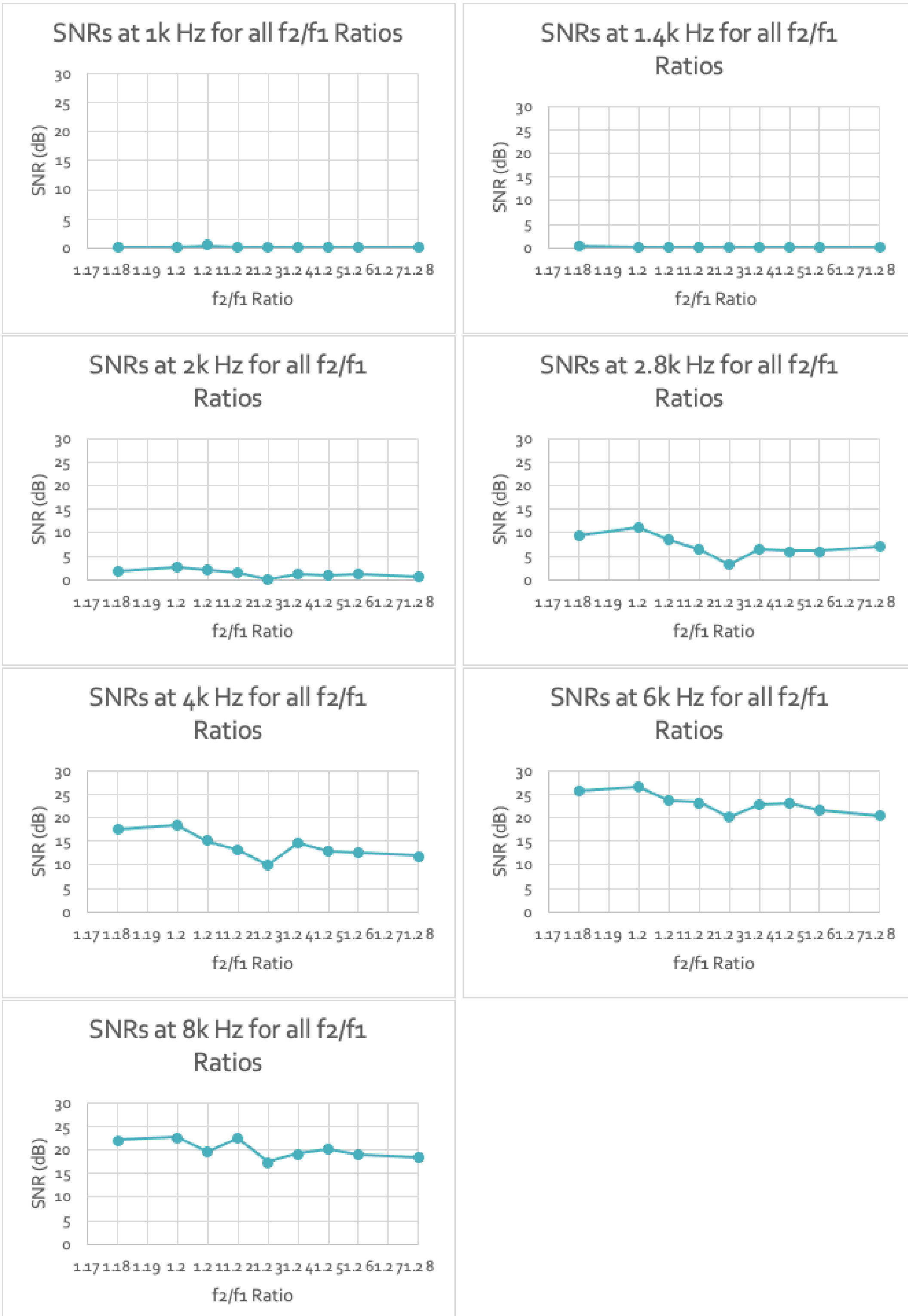


Figure 12

Signal-to-Noise Ratio Values at Individual Frequencies for All f2/f1 Ratios



An analysis of variance was run to evaluate the statistical significance of signal-to-noise ratios across frequencies for the f2/f1 ratios tested (see Table 7 for analysis results).

Table 7

Signal-to-Noise Ratio Results from the Repeated Measures Analysis of Variance using Pillai's Trace for Ratio and Frequency

Effect	Value	F	Hypothesis df	Error df	Sig
Ratio	0.863	1.574	8.000	2.000	0.445
Frequency	0.932	9.151	6.000	4.000	0.025

Results showed statistically significant differences in DPOAE SNRs across frequencies. The version of SPSS used was IBM SPSS Statistics, Version 25. Repeated measures ANOVA revealed a *p*-value of .025 ($p < .05$). The tables and figures above included absent responses (defined as <0 dB) for 2k Hz and below. On average, 6k Hz had the largest SNR with a mean of 23.0567 dB, followed by 8k Hz with a mean SNR of 20.0989 dB. These results were depicted in Figure 10.

Figure 11 depicted the SNRs at individual frequencies for all f2/f1 ratios. The SNRs at 1k and 1.4k Hz indicated absent responses. The SNRs at the frequencies of 2k Hz and above increased overall as the frequency increased with a maximum at 6k Hz. The f2/f1 ratio 1.23 had a smaller SNR at the frequencies 2k Hz and above when compared to the other f2/f1 ratios tested. The f2/f1 ratios 1.18 and 1.20 showed the largest SNRs for the frequencies of 2k Hz and above with a mean SNR of 15.33 and 16.30 dB, respectively, shown in Table 8.

Table 8

Mean Signal-to-Noise Ratio Values for 2k Hz and Above (in dB) for Each f2/f1 Ratio

f2/f1 ratio	1.18	1.20	1.21	1.22	1.23	1.24	1.25	1.26	1.28
Mean SNR (in dB)	15.33	16.30	13.83	13.37	10.20	12.95	12.62	12.12	11.70

Statistical analysis showed no significant difference among the different ratios tested with a *p*-value of .445. Analysis did show frequency to be significant ($p < .05$) at 0.003 for amplitude, 0.001 for noise, and 0.025 for SNR. This was to be expected based on what a typical DPOAE looked like. The response and noise floor would be different for different frequencies, which meant the SNR would also be different. The DPOAE responses are different for different frequencies, which was shown in the current study because responses at 1-2k Hz fell into the noise floor and higher frequencies had a large response.

A post hoc analysis with the nonparametric Kruskal-Wallis test evaluated each frequency to determine if any of the f2/f1 ratios were significantly different from the others. It was found that none of the f2/f1 ratios were statistically significantly different from any other ratios at the specific frequencies of 2.8k, 4k, 6k, and 8k Hz with *p*-values at 0.989, 0.923, 0.883, and 0.986 ($p < .05$), respectively.

CHAPTER V

DISCUSSION AND CONCLUSIONS

Research Questions, Hypotheses, and Data

Q1 When measuring distortion product otoacoustic emissions (DPOAEs) in canines, does the amplitude pattern across frequencies for the 2f1-f2 distortion product change with changes in the f2/f1 ratio?

H1 The amplitude patterns across frequencies for the 2f1-f2 distortion product will vary with changes in f2/f1 ratio for canine DPOAEs.

It was hypothesized that the amplitudes of the 2f1-f2 distortion product at the f2 frequencies would vary with changes in the f2/f1 ratio. Results from the study suggested the amplitudes of the 2f1-f2 distortion products did not change across frequencies with changes in the f2/f1 ratio. Figure 10 illustrated that for all f2/f1 ratios tested, 6k Hz had the largest amplitude response, followed by 8k Hz. The responses below 6k Hz decreased as the frequencies decreased. There were minimal responses at the low frequencies, 1k Hz and 1.4k Hz. It could be concluded from this study that the amplitude patterns of the distortion products did not change significantly with changes in the f2/f1 ratio for ratios between 1.18 and 1.28.

Q2 When measuring distortion product otoacoustic emissions (DPOAEs) in canines, do specific f2/f1 ratios produce different overall amplitudes of the 2f1-f2 emission?

H2 Changing the f2/f1 ratio produces different overall amplitudes of the 2f1-f2 emission.

It was hypothesized that changing the f2/f1 ratio would produce different overall amplitudes of the 2f1-f2 emission. Results from the study indicated that although there were differences in the amplitudes depending on the f2/f1 ratio, the differences were not statistically significant. Figure 10 provided a visual representation of the average SNRs for each f2/f1 ratio tested and the ratios 1.2 and 1.18 produced the largest amplitude 2f1-f2 emissions across all frequencies tested. Although Figure 10 indicated there were differences in amplitude when a Pillai's Trace multivariate test was performed, the difference in the amplitudes was not significantly different when comparing the nine ratios tested in the study. It could be concluded from this study that f2/f1 ratios between 1.18 and 1.28 did not produce significantly different DPOAE elicitations of the 2f1-f2 emission in canines.

Canine Hearing Frequency Range

The range of audible frequencies for canines is approximately 67 Hz to 45 kHz, which is a much greater range than the range for humans at approximately 67 Hz to 23 kHz (Strain, 2012). Canine cochleae have approximately 3¼ turns whereas human cochleas have on average 2¾ turns (West, 1985). The number of turns in the cochlea is related to the range of audible frequencies and basilar membrane length is related to the limits of hearing on each end of the range (West, 1985). Increasing the number of turns in the cochlea could potentially increase the octave range the mammal can hear (West, 1985), which is illustrated through the fact that canines have more turns in their cochleas than humans and they have a larger range of audible frequencies. The accepted f2/f1 ratio for humans of 1.22 has to do with the anatomical properties of the human auditory system, specifically the basilar membrane and the number of turns of the cochlea as well as the frequency range of audibility. Canines have a longer basilar membrane and a larger number of turns of the cochlea as well as a larger range of audible frequencies. Therefore, it stands to reason the f2/f1 ratio that would produce the most robust DPOAE responses for canines would be different and likely larger than the accepted ratio of 1.22 for humans.

It appeared the canine DPOAE responses were robust at all ratios tested for 4k Hz through 8k Hz, which suggested the f2/f1 ratio did not affect clinical interpretation of results. Based on the results with a small sample size, the ratio that produced the largest overall DPOAE response was 1.20. It was unexpected that the f2/f1 ratios 1.20 and 1.18 would yield the largest SNR values across the frequency ranges tested with a mean SNR of 16.302 and 15.33 dB, respectively. The ratios 1.21 through 1.28 had a mean SNR range of 10.204-13.828 dB. Figure 11 demonstrated SNR responses by frequency for each f2/f1 ratio tested and Figure 12 illustrated the SNR responses for each f2/f1 ratio at each frequency. The ratios 1.20 and 1.18 had the largest SNRs across all frequencies with clinically significant responses (above 2k Hz). Although 1.20 and 1.18 had the largest SNRs across frequencies, the results were not statistically significant when comparing SNRs of different ratios.

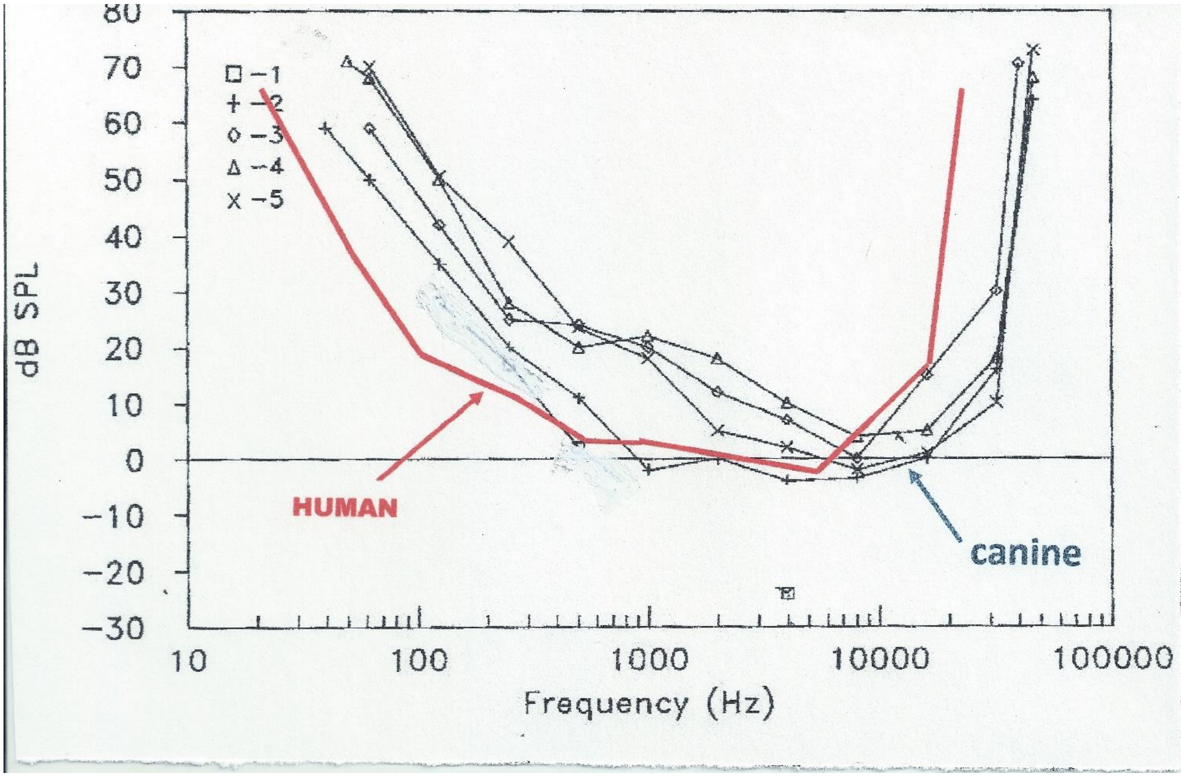
The f2/f1 ratio range was chosen because 1.22 is the typical f2/f1 ratio utilized for collecting DPOAEs in humans and due to knowledge of canine anatomy and audible frequency range, it was predicted the ratio producing the most robust responses would be larger but not excessively so. The f2/f1 ratios below 1.18 were not measured in this study due to the hypothesis that larger ratios would produce larger amplitudes because of the larger frequency range of hearing in canines and that the ratios would not differ from the accepted ratio for testing humans to an extreme degree. In addition, it was of utmost importance to balance the amount of test time per canine with the priority of conducting testing thoroughly as possible.

Brainstem auditory evoked response testing is currently the accepted hearing sensitivity assessment by the Orthopedic Foundation for Animals (Sims et al., 2017). Brainstem auditory evoked response toneburst results display excellent responses at 4k Hz through 8k Hz and above (Ter Haar et al., 2002), the same frequencies at which the current study found the most robust

DPOAE responses. The DPOAE SNRs at 2k Hz, 1.4k Hz, and 1k Hz were considered absent, which might have been due to the high noise floor; however, it was possible the canine response was not as robust at those lower frequencies as it is in humans. At the very low frequencies within the audibility frequency range for canines, hearing sensitivity is reduced (Ter Haar et al., 2002), which might account for the responses for BAER testing and DPOAE testing being reduced at the low frequencies. Figure 13, a diagram with the minimum audibility curve for humans and canines from Scheifele (2020), depicts the behavioral responses of canines and humans to pure-tone stimuli of varying frequencies. The figure illustrates the minimum intensity levels canines and humans hear and respond to across the range of frequencies. The greatest sensitivity appears to be in the range of 4k to 10k Hz for canines. The greatest sensitivity occurs in the range of 1k to 4k Hz for humans. As the frequencies decrease below 2k Hz, canine hearing sensitivity is reduced compared to that of humans. The DPOAE results from this study corresponded to the minimum audibility curve because the most robust DPOAE responses were in the range of greatest hearing sensitivity seen on Figure 13 (Scheifele, 2020) and the responses were significantly reduced or absent at 2k Hz; below this point was where hearing sensitivity was significantly reduced in canines.

Figure 13

Human and Canine Minimum Audibility Curve (Scheifele, 2020)



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

Toneburst BAER results and canine minimum audibility curves agreed with behavioral studies of canine hearing sensitivity (Ter Haar et al., 2002). The DPOAE results from the current study were in agreement with the toneburst BAER results and canine minimum audibility curves, which led us to conclude the DPOAE results were in agreement with the behavioral studies and BAER test results.

All the canines exhibited similar DPOAE frequency responses; 2k Hz and below had extremely poor results with amplitudes peaking at 6-8k Hz. This indicated the frequencies of best responses for canines were higher than those measured for humans since DPOAE responses for humans tend to peak in the mid-frequencies of 1.5k to 6k Hz (Gorga et al., 1997), with the best responses in the range of 2-4k Hz. The differences in frequency response of DPOAEs for canines and humans might relate to the differences in audible hearing range, basilar membrane length, and number of turns in the cochlea.

The canine DPOAE response amplitudes are much larger than the typical DPOAE amplitude in human adults. The amplitudes are more like human infant DPOAE responses. Abdala and Keefe (2006) reported as much as a 13 dB difference in DPOAE response amplitude for infants when compared to adults. The difference tended to decrease with an increase in age. Stamate et al. (2015) reported infant DPOAE responses were larger at high frequencies than adult responses. This corresponded to the responses found in the current study as DPOAE responses were overall larger in amplitude than adult human responses, especially in the high frequencies. The canine DPOAE responses in the current study were more like infant human DPOAEs than adult human DPOAEs.

Study Limitations

A limitation of this study was the lack of a large sample size that included a variety of dog breeds, sizes, genders, and ages. In the current study, no attempt was made to control for size (the only limitation being the size of available Mutt Muffs (Safe & Sound Pets, 2021), breed, and gender. However, the range of SNRs for the 10 subjects was quite small, especially at 6k Hz and 8k Hz. Age was limited to a range of 2-8 years old; however further research should be conducted if age normative data are to be established. The subjects in the study varied in size, age, breed, and gender. However, due to a small sample size, it could prove useful to have additional testing to determine if there are variations of responses with respect to those factors. Taking into consideration that the subjects did vary in size, breed, age, and gender, it would not be expected that there would be a significant difference in responses or that these results would be different for canines with these different factors.

In addition, it is known that multiple distortion products are produced in mammalian ears including those of humans. It has been established that in humans, the distortion product with the largest amplitude is 2f1-f2 (Christensen et al., 2015). It is possible that canine ears might respond differently to probe frequencies f1 and f2, producing distortion products at other mathematical combinations that are larger than those seen in human ears. Further study is needed to determine if other distortion products are present in canine ears at levels that vary compared to those of humans.

Prior to using OAEs clinically to aid in diagnosis of hearing status in canines, further research needs to be conducted to standardize testing procedures, equipment modifications, and software settings that would produce optimal OAEs in canines.

Test Environment/Use of Mutt Muffs

The lack of a probe that fits a canine ear canal was an additional limitation. It might be beneficial to develop a canine probe if DPOAEs are to be used clinically in veterinary offices as a screening tool. Further studies are necessary to determine if there are effective methods to reduce ambient noise and/or canine internal noise that could impact DPOAE test results. Mutt Muffs (Safe & Sound Pets, 2021) provided adequate ambient noise reduction to collect DPOAE responses in the subjects for this study; however, it is still unknown if the Mutt Muffs would provide enough ambient noise reduction in a veterinary clinic for DPOAEs to be clinically useful. In addition, the Mutt Muffs might have potentially created resonances that influenced the frequencies of the DPOAE responses. Because of this, it is recommended that further research be conducted to determine if Mutt Muffs impacted DPOAE responses by creating resonances while reducing the ambient noise reaching the ear canal.

Some potential benefits to creating a probe specifically designed for canine ear canals include:

- Less equipment necessary for veterinary clinics to keep on hand to perform hearing screenings utilizing DPOAEs. There are multiple sizes of Mutt Muffs (Safe & Sound Pets, 2021) to accommodate the large variation of head size in canines so a veterinary clinic would need to purchase all available sizes of Mutt Muffs.
- A better seal might reduce ambient noise more than Mutt Muffs due to the fact that it would completely block the ear canal, whereas Mutt Muffs might not always fit or be placed correctly.
- Less user error would occur when the only piece of equipment necessary to place is the sealing probe instead of a probe that does not fit and seal correctly in addition to the Mutt Muffs.
- Any potential for a resonance effect with the Mutt Muffs on is eliminated when only a specifically designed probe for canine ear canals is used.

Some benefits of utilizing Mutt Muffs (Safe & Sound Pets, 2021) that might mean a probe specifically designed for canine ears is not necessary:

- The Mutt Muffs reduced ambient noise adequately to collect DPOAEs, meaning the effort to develop a special probe might be superfluous.
- The Mutt Muffs served a purpose in that all 10 subjects of the study were noted to be much calmer and more compliant during testing when the Mutt Muffs were secured over their ears.

- The Mutt Muffs also prevented the canines from being able to pull the probe out of their ear canal by pawing at their ear or shaking their head.
- The lack of a correctly fitting and sealing probe seemed to be compensated by the use of Mutt Muffs.

The noise floor cutoff was adjusted to 10 dB to allow for consistent data collection. It was determined to be unrealistic to have the cutoff noise floor intensity be at 5 dB SPL, specifically for the lower frequencies tested (2k Hz and below). The noise floor was consistently between 5-15 dB SPL for 2k Hz and below even when the noise floor was below 5 dB SPL for the frequencies above 2k Hz. Data collection was continued when the DPOAE responses and the noise floor were at or below 10 dB SPL for the frequencies above 2k Hz. Although each canine was in the sound booth with the door securely closed, the Mutt Muffs (Safe & Sound Pets, 2021) were on and secure, and all ambient noise was reduced or eliminated, there was significant noise for the lower frequencies. It is hypothesized that the increase in lower frequency noise might have been due to the canine's internal noise (i.e., breathing, panting, swallowing, licking). Extensive efforts were made to keep the canines calm and settled to reduce the noise as much as possible. Results from subject 2 (see Appendix B) illustrated the additional noise, in dB SPL, that occurred when the canine was consistently panting. Although efforts were made to reduce the occurrence of panting during testing, the subject would pant whenever the DPOAE tests were being conducted. The canine was approximately 4-years-old at the time of testing, had no risk factors for otologic issues, and subjectively responded appropriately to sounds. It should be noted that the results from this subject might have been skewed because of the amount of noise present during testing.

It was interesting to note that for all subjects tested, there was an inability to record DPOAE responses in the frequency range below 2k Hz. This might be due to the canine's internal noise such as breathing, panting, and swallowing. It might also be that canines just do not have robust DPOAE responses below 2k Hz because even when the noise floor was low (ambient noise level in the empty sound-treated booth measured with sound level meter to be 40.3 ± 1.5 dBA in the frequency range 31.5 Hz – 8k Hz) and allowed for collection of responses at all frequencies tested (i.e., subject 4, f2/f1 ratio: 1.20), there was a consistent lack of responses across all 10 subjects for the frequencies below 2k Hz regardless of the f2/f1 ratio tested.

Recommendations for Clinical Use of Distortion Product Otoacoustic Emissions with Canines

1. Clinical use of DPOAEs on canines at this time should strictly be for hearing screening. Distortion product otoacoustic emissions results should not be interpreted as hearing test results; if there is a lack of robust responses, then further evaluation, such as BAER testing, should be performed to obtain more conclusive results of hearing status. This was a great screening tool because it took under five minutes total, was non-invasive, could be performed while the canine was awake and alert, and could provide ear specific screening results for each canine patient. Correlation with audiometric data might be done in the future.
2. A sound-treated booth was not necessary; however, it would be beneficial if available. For the purpose of using DPOAEs in a veterinary clinic, a sound-treated booth is likely not available. This does not mean veterinary clinics cannot utilize DPOAEs to screen their canine patients. One recommendation would be for veterinary clinics to attempt to find a quieter location within their clinic to try to reduce ambient noise as much as possible.

3. Distortion product otoacoustic emissions screening might possibly be performed without Mutt Muffs (Safe & Sound Pets, 2021); however, without a specially designed probe for canine ear canals, a veterinary clinic might have too much ambient noise for responses to be collected without Mutt Muffs. Mutt Muffs provided that noise reduction and helped to secure the probe in place, both of which might be useful in a veterinary clinic setting.
4. It is recommended to have at least two testers when performing DPOAEs on a canine. A set up with two testers is recommended as follows: one person to run the DPOAE screening tool (computer, handheld screener) and one person to place the probe and then the Mutt Muffs who can restrain or keep the dog calm and compliant throughout testing. If this is being performed in a veterinary clinic and the owner is present, it is ideal if the owner can sit with the canine to keep them calm and compliant.
5. The results from this study indicated the canine must be relatively quiet, calm, and compliant. When one of the canines was panting throughout testing, it was difficult to obtain robust responses. The canine should be sitting or lying down, be as still as possible, and should be comfortable to avoid panting. Keeping the dog from licking, drinking, eating, and making other unnecessary noise is imperative. Some of the techniques utilized to help keep the canines quiet were to (a) have the owner there and providing calm support for the canine, (b) recommend the canines get some exercise prior to the testing (but not immediately before since that would increase likelihood of panting), (c) have the owners bring treats to help increase compliance

if needed, (d) provide water, and (e) allow the canine to explore the room for approximately 5-10 minutes prior to testing to familiarize them with the space.

6. The frequency ratios recommended as a result of this study would be ratios in the range of 1.18-1.22. Although the f2/f1 ratios that produced the most robust responses were 1.18 and 1.20, there was no statistically significant difference between any of the f2/f1 ratios utilized in this study. The lack of significance between ratios suggested that any of the ratios would be adequate; however, because 1.18 and 1.20 did have the most robust responses, it would be ideal to use ratios within that range. The ratio 1.22 is typically utilized for DPOAEs in humans and due to the robust responses with this ratio, it would be acceptable to use 1.22 at this time especially if that aided in ease of use.
7. The results from the study suggested a certain range of frequencies should be focused on to determine a pass/fail for screening purposes. Due to the consistent lack of responses below 2k Hz, it is recommended the screening guidelines for a pass/fail would focus on frequencies at and above 2k Hz. This would especially be true if the screening was being performed in a clinic environment where there would potentially be greater environmental noise, further reducing results below 2k Hz even more. The screening could include frequencies below 2k Hz; however, the pass/fail criteria are not recommended to be based on those frequencies as it would likely cause a high percentage of canines to have false positive results. This means DPOAE instrumentation designed for use with humans should be modified to avoid false positive results when canine responses are not present below 2k Hz.

8. Future research is a necessity if DPOAEs are to become a clinical screening tool for canines' hearing status. Studies of a greater scale and controlled for age, size, breed, and gender would provide greater insight into the expected results for DPOAEs in canines with normal hearing sensitivity. If this tool is to be used clinically, research would need to be conducted to provide normative values for all canines. Additionally, it would prove useful to create canine probe tips and an earmuff system to secure and reduce ambient noise in the environment that could be used in clinics. Standardizing test procedures and settings on software that would produce optimal DPOAE results is also recommended.

Conclusions

Distortion product otoacoustic emissions were successfully elicited on all 10 canine subjects in the study with Mutt Muffs (Safe & Sound Pets, 2021) on in a sound-treated booth. The DPOAE responses were consistent across the different ratios tested for each canine. From the results of the study, it could be concluded that DPOAEs could be elicited from the canine ear.

If DPOAEs are to be used clinically as a screening tool in veterinary clinics, they must be a relatively simple, quick, and non-invasive procedure. Some considerations regarding the clinical usefulness of DPOAEs are that veterinary offices are spaces with greater ambient noise than a sound booth, the canine must be relatively calm and settled, and the use of Mutt Muffs (Safe & Sound Pets, 2021) might be necessary to help reduce the ambient noise. Even with the extensive efforts of ambient noise reduction in the study, there were typically high levels of noise for the lower frequencies. This was hypothesized to be due to internal noise such as the canine's breathing, panting, swallowing, licking, etc. When adding the internal noise to the ambient noise of a veterinary clinic, there is potential that the DPOAE responses would be masked by the noise.

The purpose of this study was to determine what stimulus frequency ratios would produce the most robust OAEs in canines to support future clinical use of OAEs to aid in diagnosis of hearing status in canines. The results from the study indicated f2/f1 ratios between 1.18 and 1.28 did not produce significantly different DPOAE elicitations of the 2f1-f2 emission in canines and the amplitude patterns of the distortion products did not change significantly with changes in the f2/f1 ratio for ratios between 1.18 and 1.28.

Based on results from the study, it was concluded the typical canine DPOAE response was 2k Hz and above. Lack of DPOAE responses below 2k Hz regardless of the noise floor levels, low ambient noise, and level of canine compliance and degree of quietness suggested the typical DPOAE response was best at 2k Hz and above. In general, the canine DPOAE responses were robust enough that the f2/f1 ratio did not seem to significantly impact the overall amplitudes. Due to the lack of a probe specifically designed for canine ears, use of Mutt Muffs (Safe & Sound Pets, 2021) or a similar device is recommended; however, further research is necessary to determine if resonance under the Mutt Muffs changed responses.

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APPENDIX A

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL

IACUC Memorandum

To: Jennifer Weber

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

CC: Kathryn Bright, Sara Hartson and IACUC Files

Date: April 3, 2019

Re: IACUC Protocol 1903C-JW-D-22 Approval

The UNC IACUC has completed a final review of your protocol "Acoustic DPOAEs in Canines: Systematic Changes in Amplitude (f2/f1 Ratio)". The protocol review was based on the requirements of Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training; the Public Health Policy on Humane Care and Use of Laboratory Animals; and the USDA Animal Welfare Act and Regulations. Based on the review, the IACUC has determined that all review criteria have been adequately addressed. The PI/PD is approved to perform the experiments or procedures as described in the identified protocol as submitted to the Committee. This protocol has been assigned the following number 1903C-JW-D-22.

The next annual review will be due before April 3, 2020.

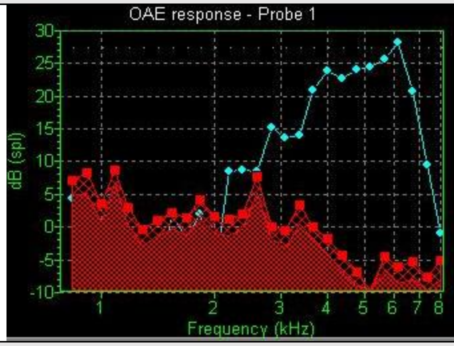
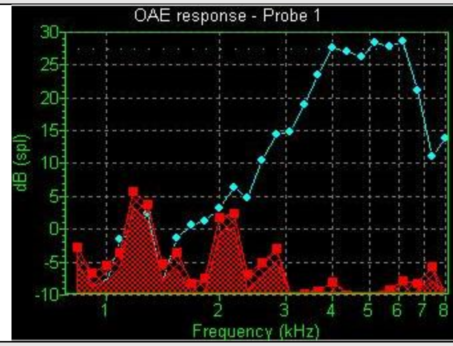
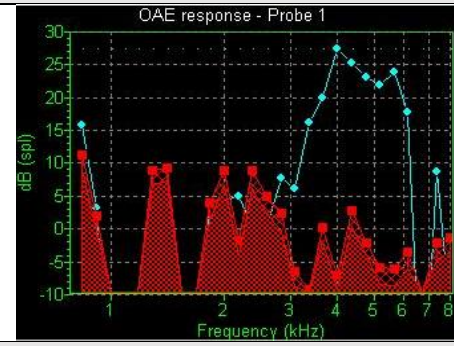
Sincerely,

Laura Martin, Director of Compliance and Operations

APPENDIX B

DISTORTION PRODUCT OTOACOUSTIC EMISSIONS LINE GRAPH REPRESENTATIONS FOR TEST SUBJECTS

SUBJECT 1

1.18	1.20	1.21
		
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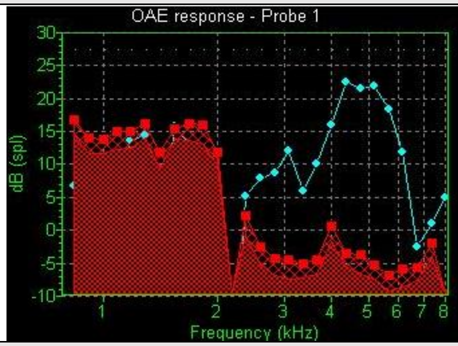
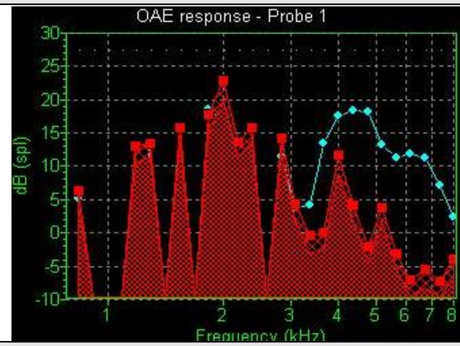
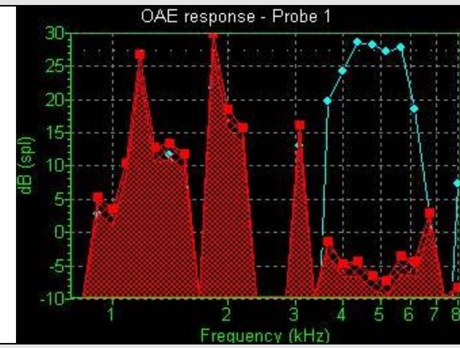
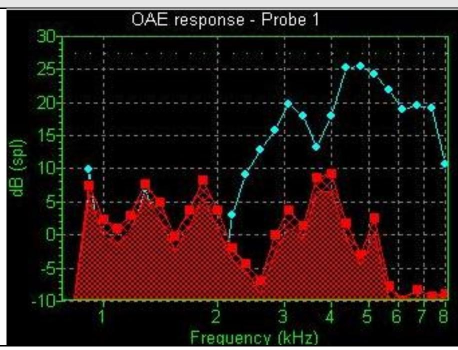
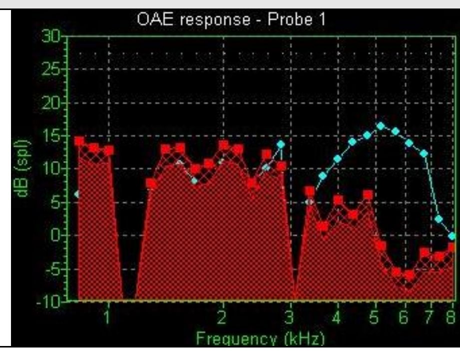
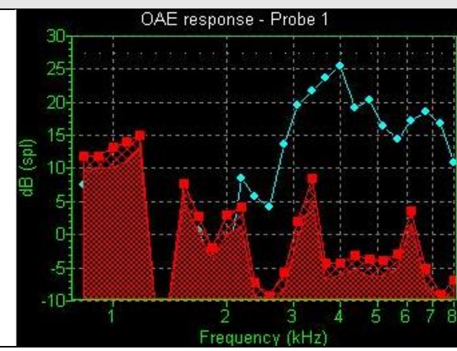
		
1.25	**1.26**	**1.28**
		

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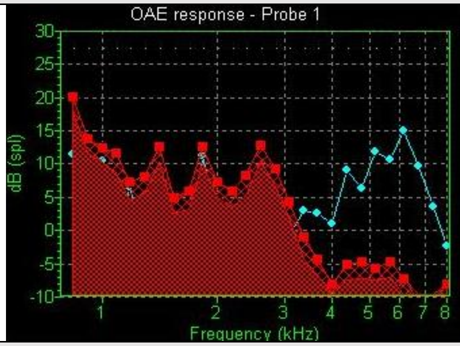	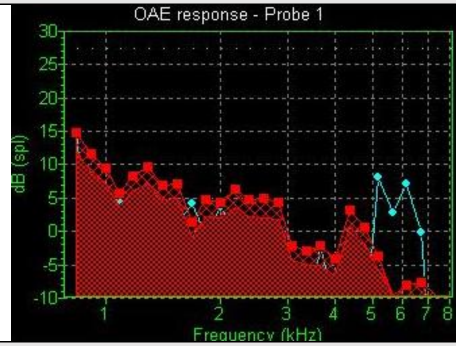	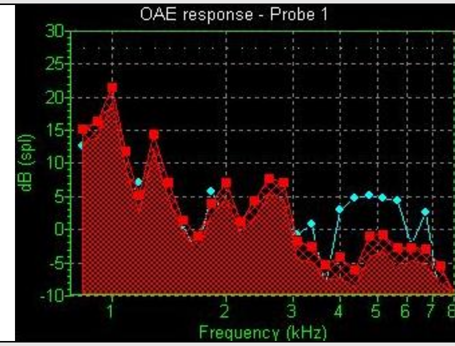
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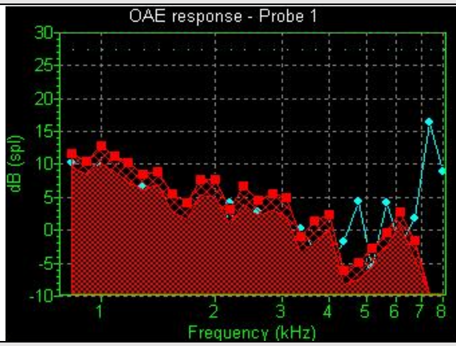	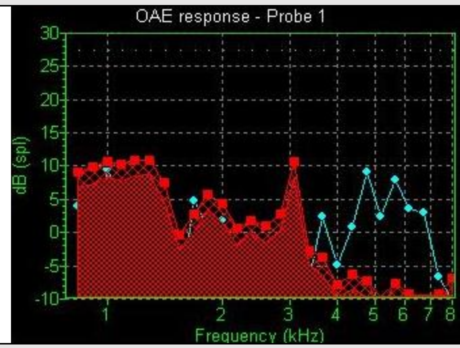	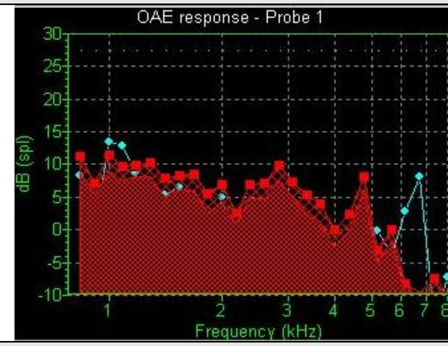
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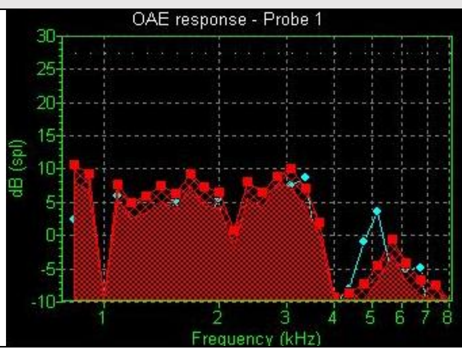	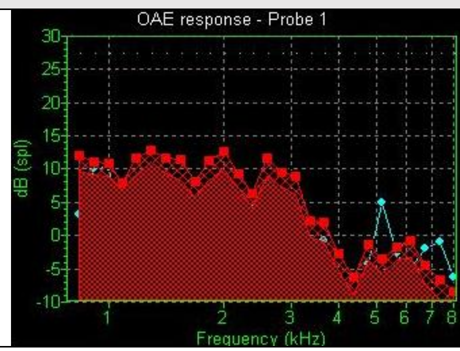	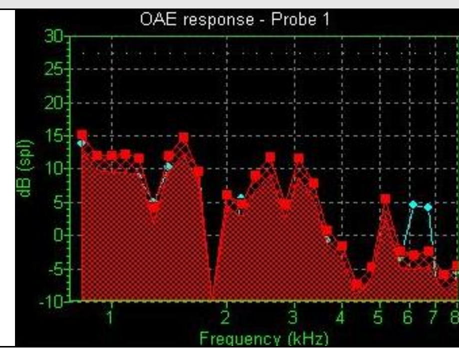

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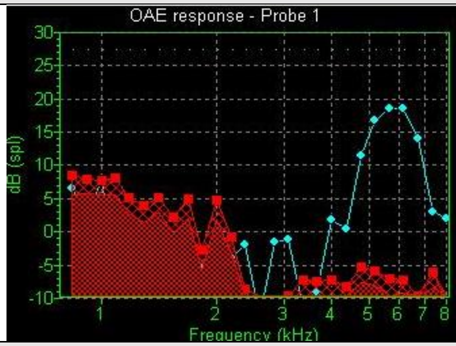

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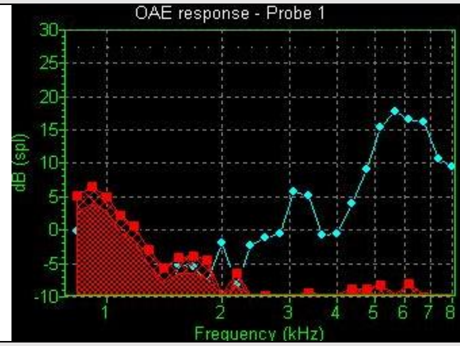

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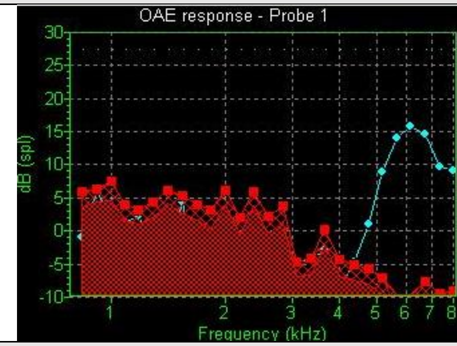

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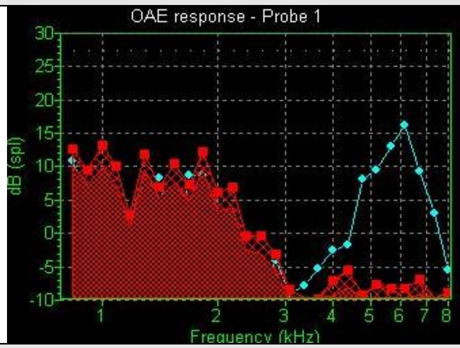

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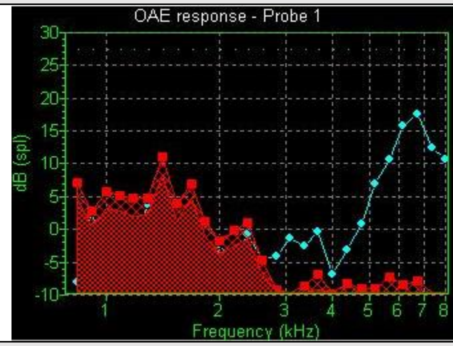

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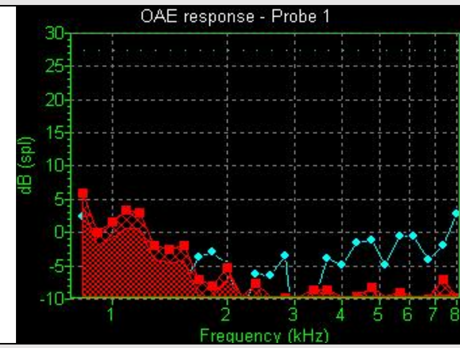

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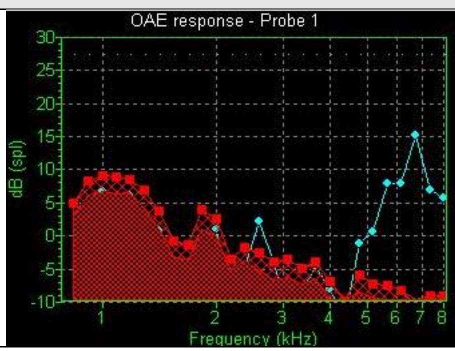

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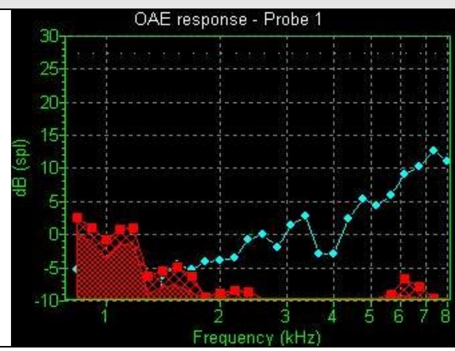

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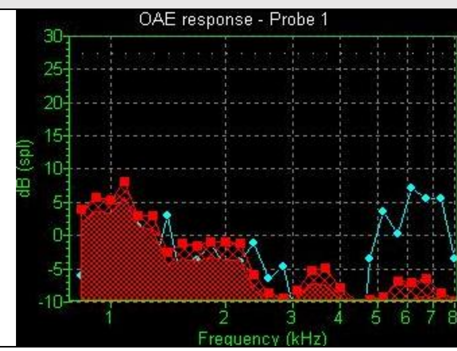

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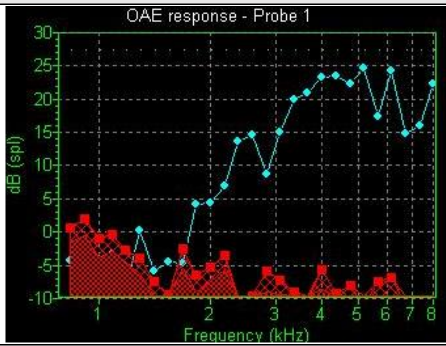	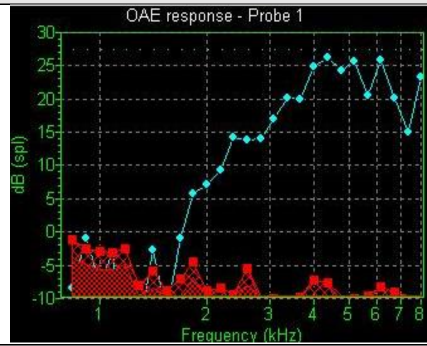	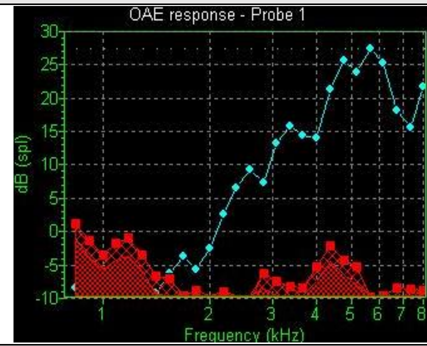
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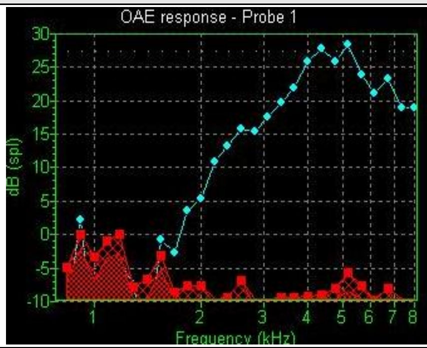	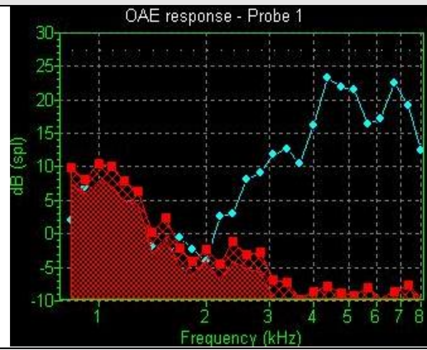	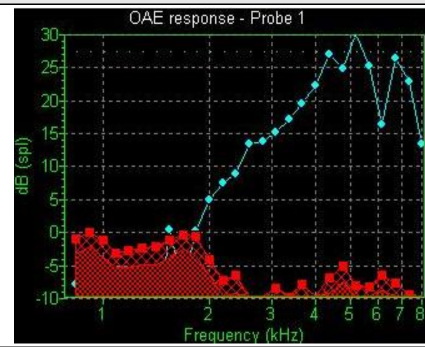
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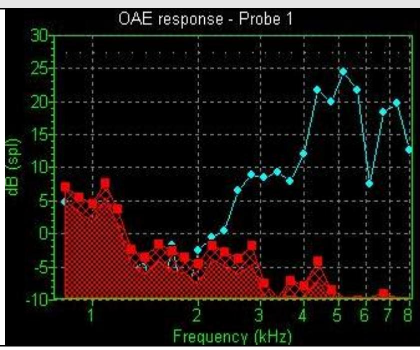	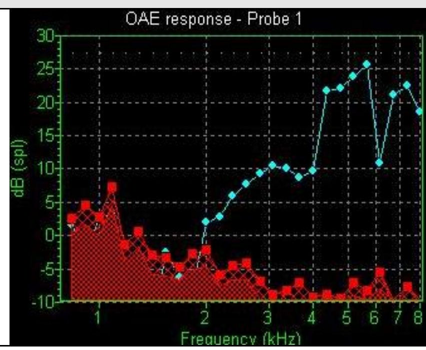	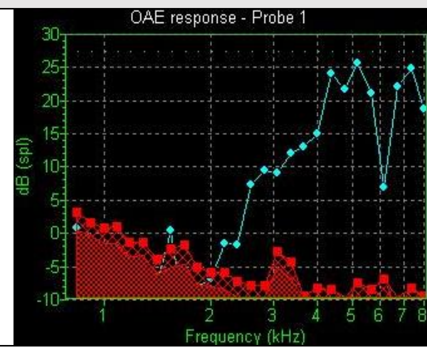

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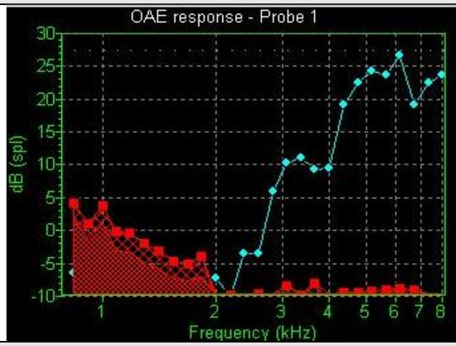

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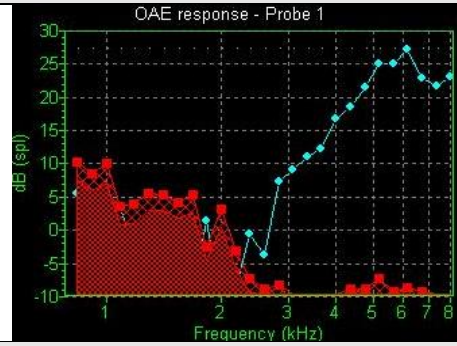

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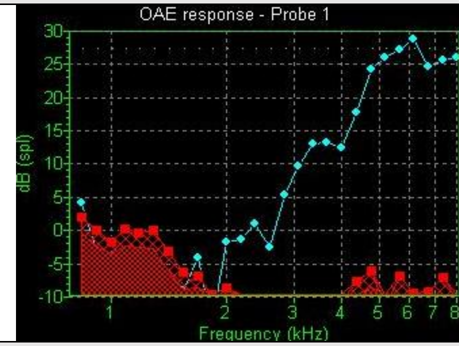

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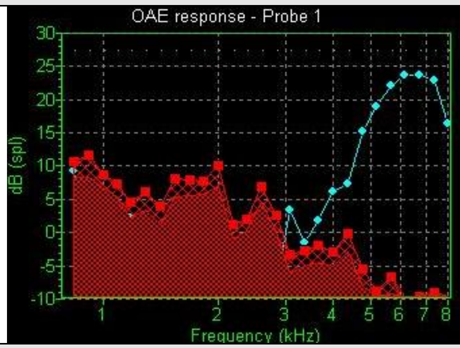

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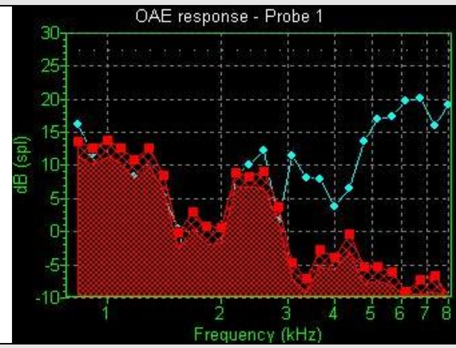

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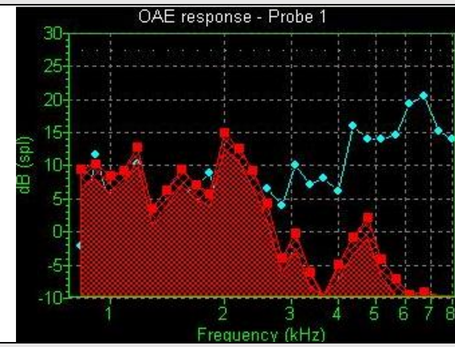

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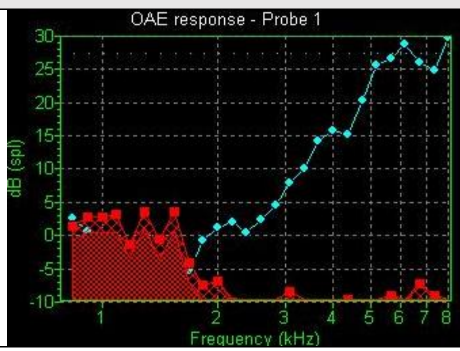

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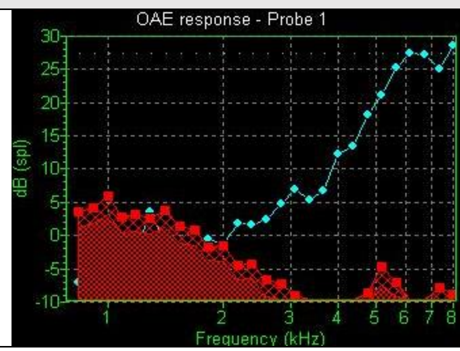

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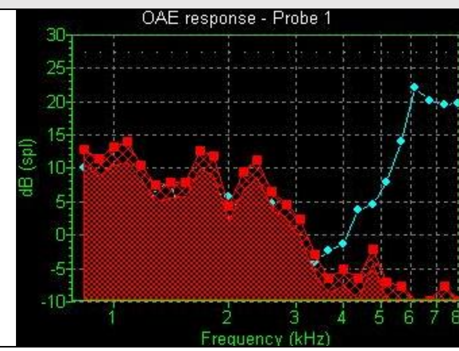

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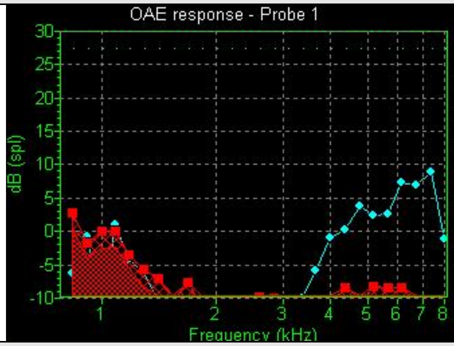

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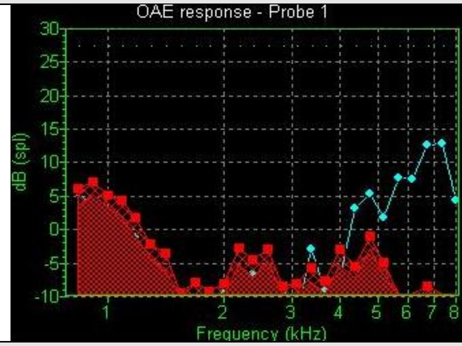

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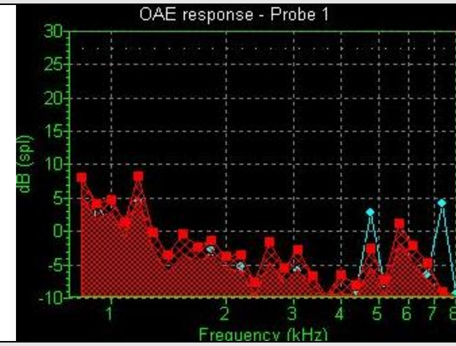

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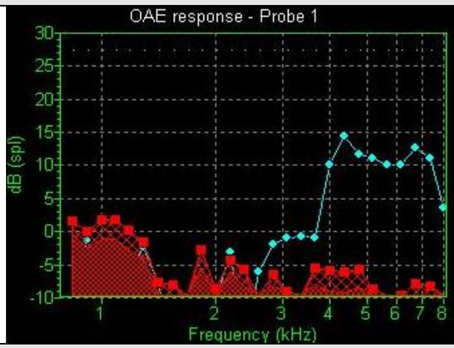

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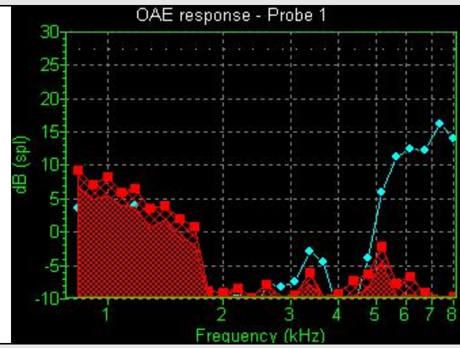

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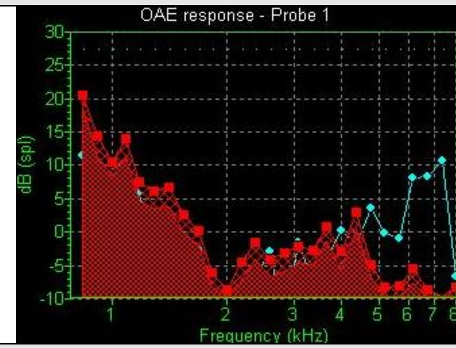

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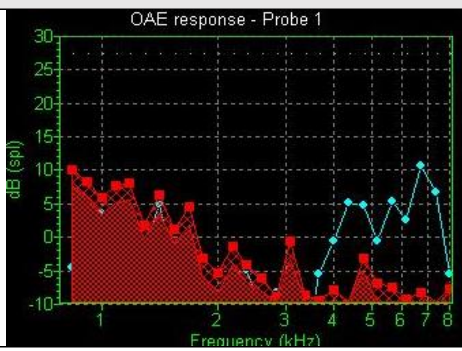

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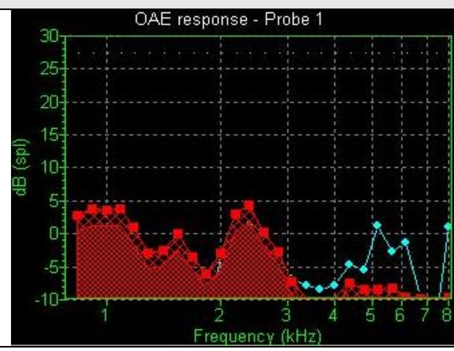

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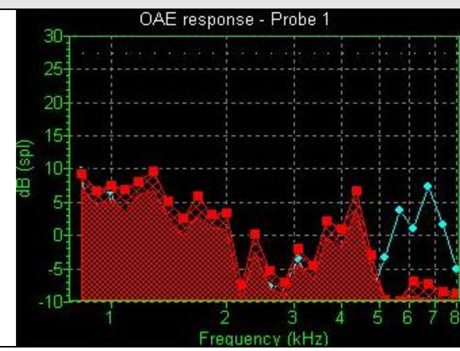

SUBJECT 7

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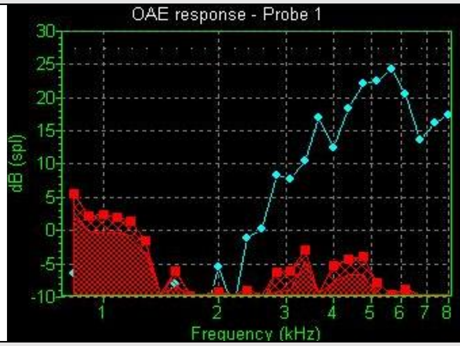
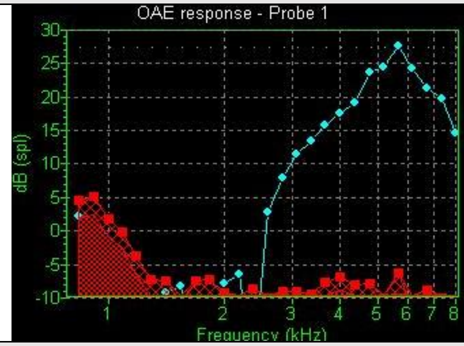
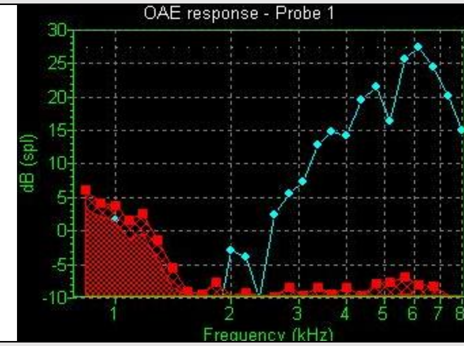
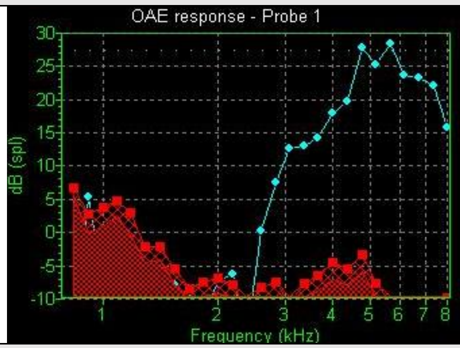
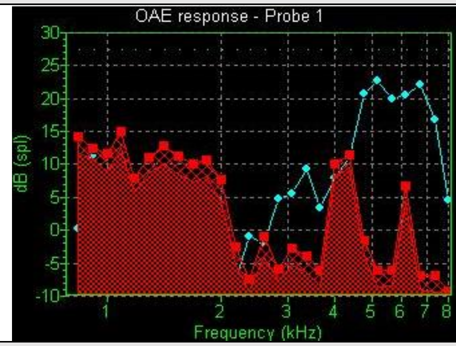
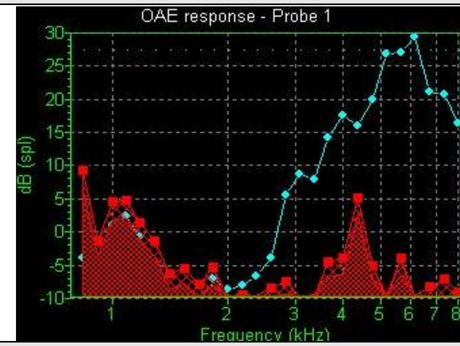
		
1.22	**1.23**	**1.24**
		
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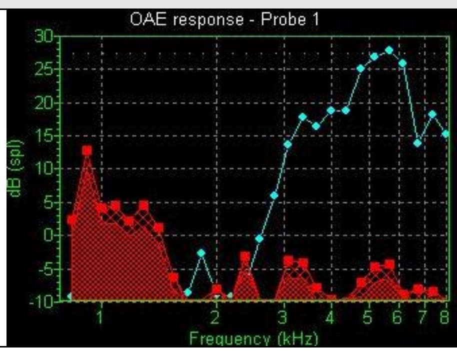	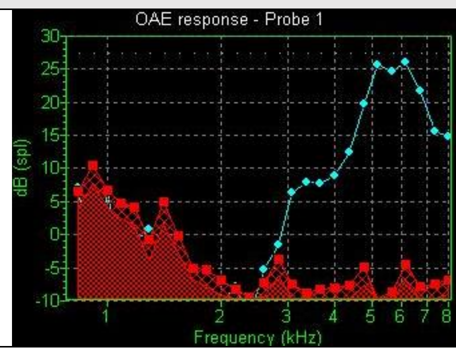	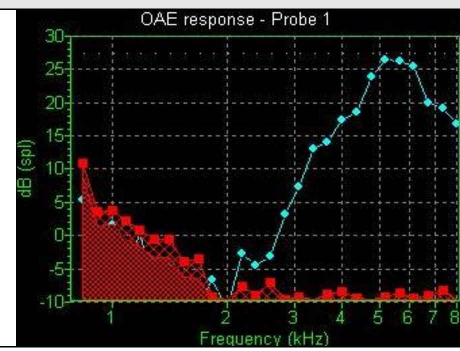

SUBJECT 8

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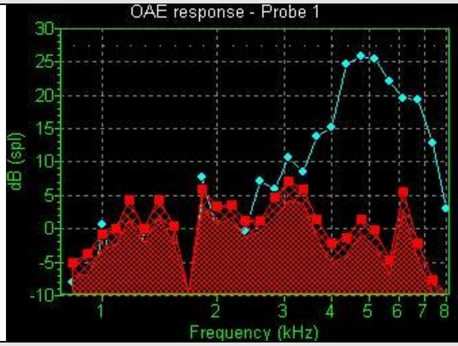
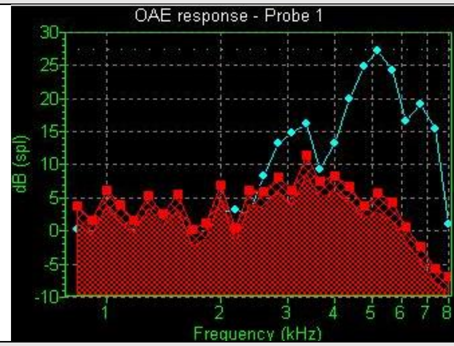
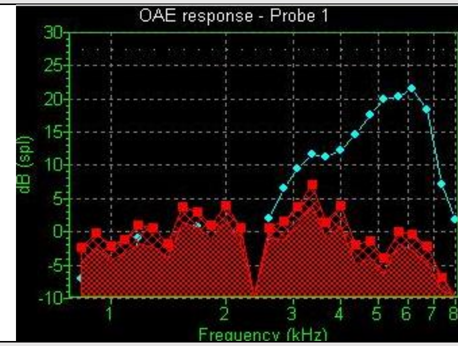
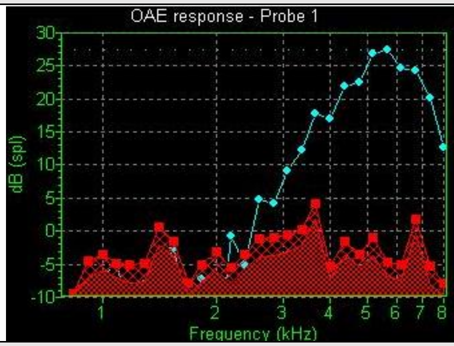
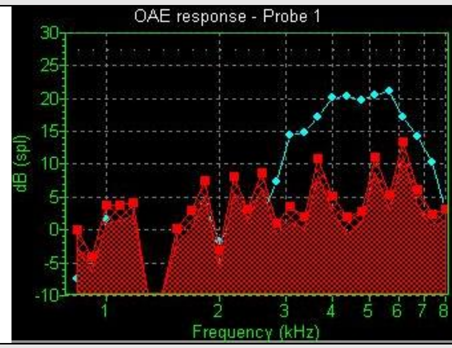
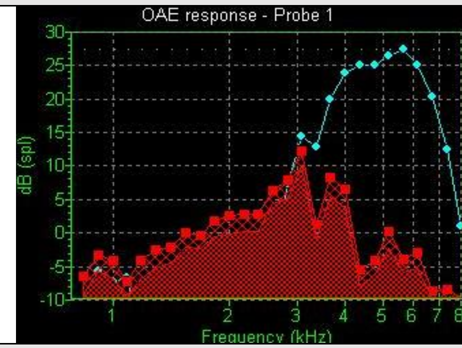
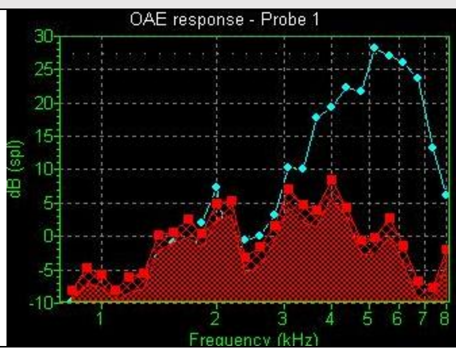
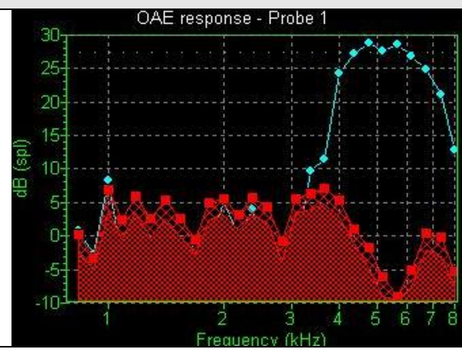
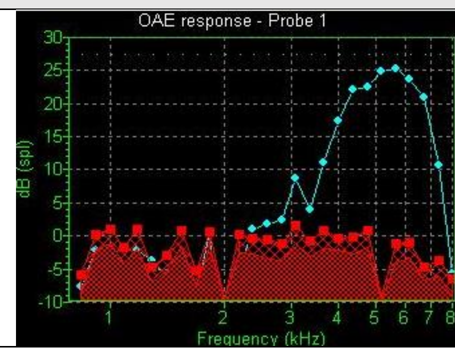
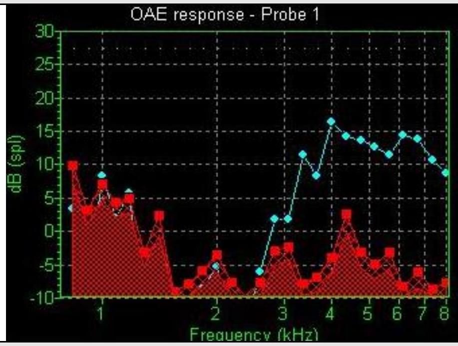
		
1.22	**1.23**	**1.24**
		
1.25	**1.26**	**1.28**
		

SUBJECT 9

1.18



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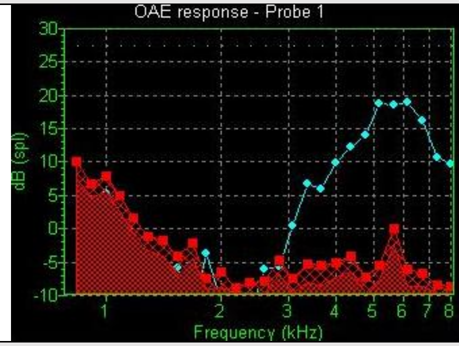

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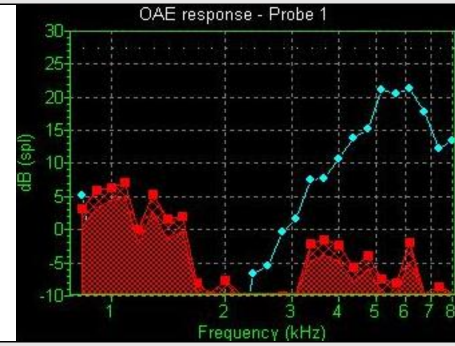

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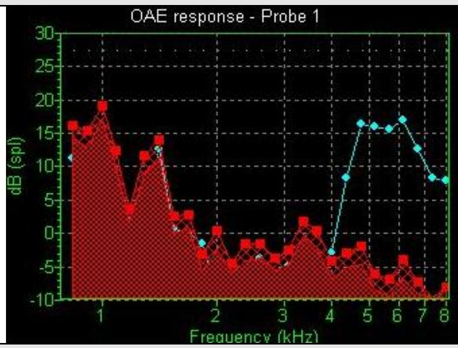

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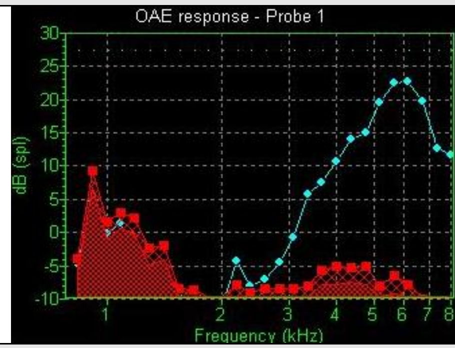

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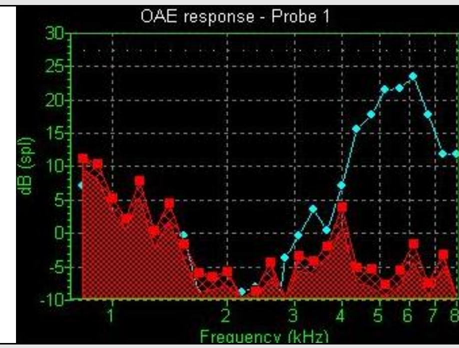

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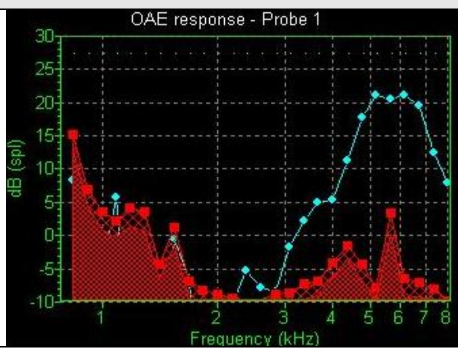

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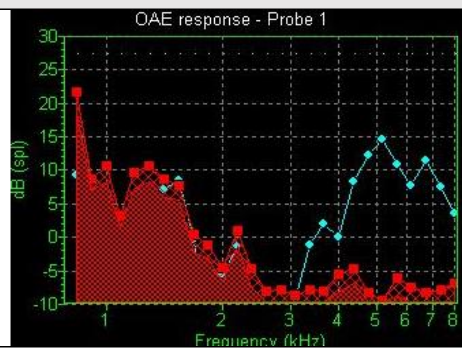

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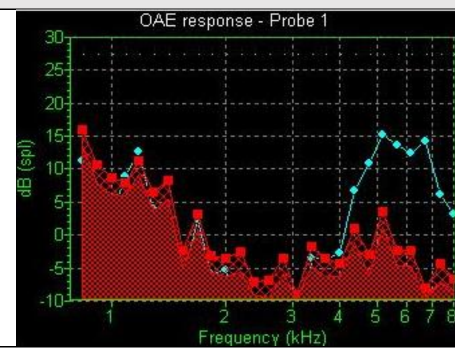

SUBJECT 10

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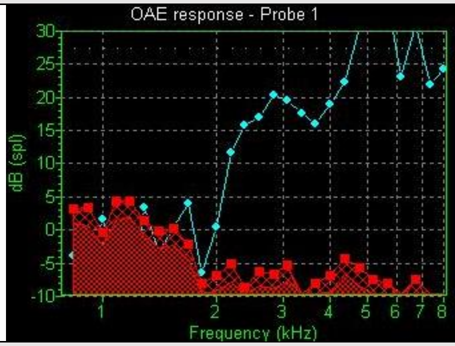

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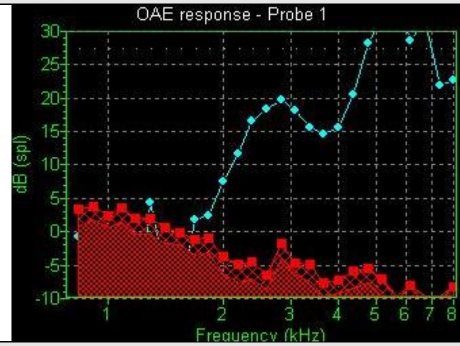

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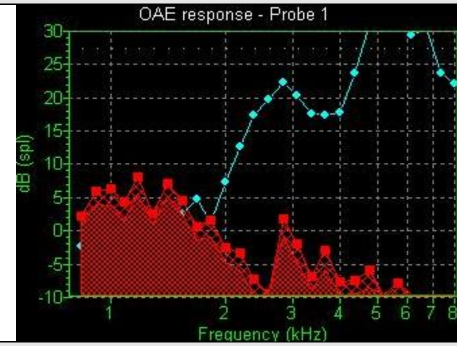

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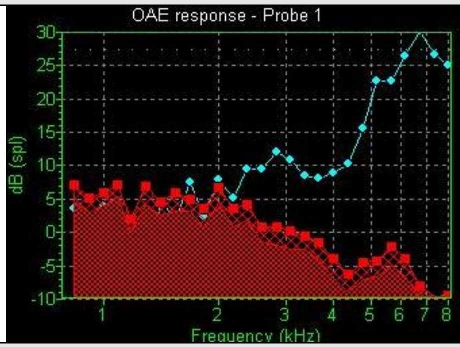

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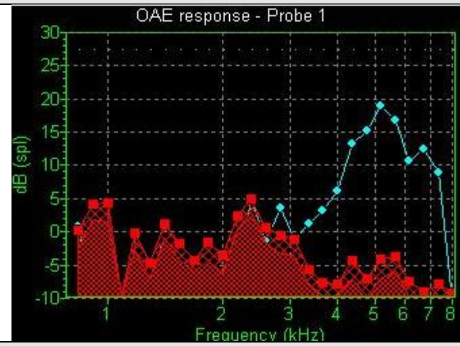

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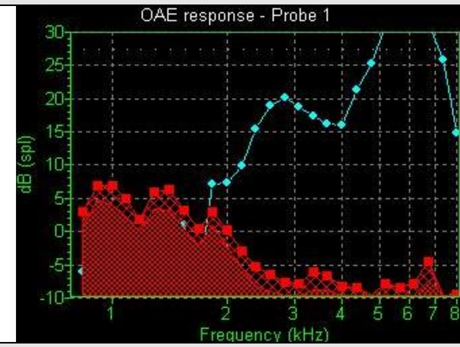

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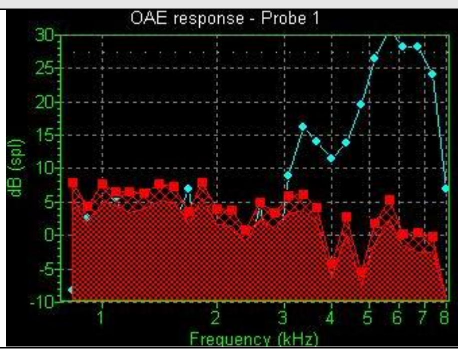

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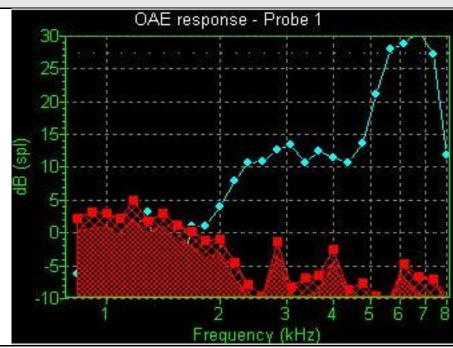

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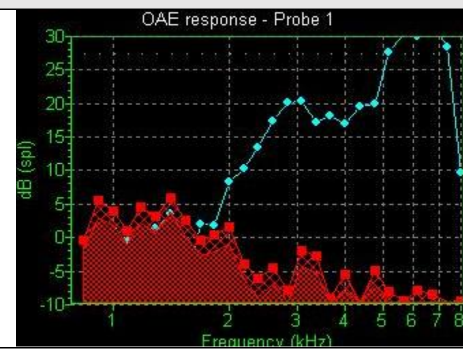

APPENDIX C

MEAN SIGNAL-TO-NOISE RATIOS FOR EACH RATIO AT EACH OF THE FREQUENCIES TESTED

Ratio	Freq	*M (dB)*	*SD*	*N*
1.18	1K	0.0000	0.00000	10
	1.4K	0.3800	1.16695	10
	2K	1.8300	3.78713	10
	2.8K	9.5200	8.75681	10
	4K	17.4900	8.39860	10
	6K	25.7300	7.92886	10
	8K	22.0800	10.13737	10
1.20	1K	0.0000	0.0000	10
	1.4K	0.0000	0.0000	10
	2K	2.7800	4.74477	10
	2.8K	11.0700	8.96971	10
	4K	18.3900	13.49728	10
	6K	26.5800	10.29539	10
	8K	22.6900	9.85128	10
1.21	1K	0.4000	1.26491	10
	1.4K	0.0000	0.0000	10
	2K	2.2000	3.44513	10
	2.8K	8.4500	8.76537	10
	4K	15.0200	10.67310	10
	6K	23.8100	12.83445	10
	8K	19.6600	12.15376	10
1.22	1K	0.0000	0.0000	10
	1.4K	0.0000	0.0000	10
	2K	1.5200	4.40121	10
	2.8K	6.5700	8.49929	10
	4K	13.0500	10.53252	10
	6K	23.2600	9.56907	10
	8K	22.4400	9.54512	10
1.23	1K	0.0000	0.0000	10
	1.4K	0.0000	0.0000	10
	2K	0.2400	0.75895	10
	2.8K	3.2800	3.66933	10

Ratio	Freq	*M (dB)*	*SD*	*N*
1.23	4K	9.9300	7.40646	10
	6K	20.1500	6.12867	10
	8K	17.4200	9.78511	10
1.24	1K	0.0000	0.0000	10
	1.4K	0.0000	0.0000	10
	2K	1.3500	2.36890	10
	2.8K	6.5900	9.71922	10
	4K	14.7600	11.15250	10
	6K	22.8800	11.98998	10
	8K	19.1700	9.83114	10
1.25	1K	0.0000	0.0000	10
	1.4K	0.0000	0.0000	10
	2K	0.9600	2.17675	10
	2.8K	6.1000	6.72524	10
	4K	12.8800	9.15312	10
	6K	22.9500	10.01435	10
	8K	20.2200	10.33148	10
1.26	1K	0.0000	0.0000	10
	1.4K	0.0000	0.0000	10
	2K	1.2600	2.01119	10
	2.8K	6.0900	6.97686	10
	4K	12.6900	7.82453	10
	6K	21.6400	11.73894	10
	8K	18.9100	11.44799	10
1.28	1K	0.0000	0.0000	10
	1.4K	0.0000	0.0000	10
	2K	0.6900	2.11263	10
	2.8K	7.0500	8.35387	10
	4K	11.9400	11.46862	10
	6K	20.5100	11.64135	10
	8K	18.3000	11.69197	10