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TEMPERATURE EFFECTS ON OTOACOUSTIC EMISSIONS AND AUDITORY BRAINSTEM RESPONSES IN RATS

by

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DOCTORAL THESIS APPROVAL PAGE

This is to certify that the thesis prepared by Amanda N. Snyder, entitled Temperature Effects on Otoacoustic Emissions and Auditory Brainstem Responses in Rats, has been approved by this committee as satisfactory completion of the requirement for the degree of Doctor of Audiology (Au.D.) in the department of Audiology, Speech-Language Pathology and Deaf Studies.

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ABSTRACT

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Otoacoustic emissions (OAEs) and Auditory Brainstem Responses (ABRs) are objective diagnostic tools used in audiology to provide information regarding pathologies of the auditory system. Several factors, besides hearing loss, can affect OAEs and ABRs. Internal factors include body temperature and internal noise of the individual, whereas some external factors include environmental noise, probe placement, and equipment parameters. The purpose of this study was to provide more information on the effects of body temperature on Distortion Product Otoacoustic Emissions (DPOAEs) and tone-burst Auditory Brainstem Reponses in the pigmented rat. Results indicate that there was no significant effect of body temperature on either the amplitude of the DPOAEs or the threshold of the tone-burst ABRs.

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CHAPTER 1

INTRODUCTION

Otoacoustic emissions (OAEs) and Auditory Brainstem Responses (ABRs) are objective diagnostic tools used in audiology to provide information regarding pathologies of the auditory system. OAEs provide information regarding the status of the middle ear and the cochlea. ABRs provide information beyond the cochlea regarding the status of the auditory nerve and the lower portion of the brainstem.

In less than 50 years, OAEs have transitioned from a primarily research-based tool to a widely used clinical tool. OAEs are created within the cochlea and can be measured with specialized equipment. Spontaneous OAEs (SOAEs) are sounds generated within the cochlea in the absence of external stimulation and evoked OAEs (EOAEs) are "echoes" that are created in the cochlea in response to a sound entering the ear (Kemp, 1998). The emissions from the ear are thought to originate from the motion of the outer hair cells (OHC) (Prieve & Fitzgerald, 2002). If the outer hair cells are damaged, OAEs are absent or reduced in amplitude (Gorga et al., 2000). The motility of the outer hair cells, first described by Davis (1983), is the active movement of the outer hair cells and is commonly called the "cochlear amplifier" (Davis, 1983; Seifert et al., 2001) because this motion serves to amplify the magnitude of low to moderate intensity sounds entering the ear. Clinically, OAEs are used as a tool to confirm pure-tone behavioral testing, detect malingering, screen for hearing in newborns, and monitor the effects of ototoxic drugs,

occupational noise exposure, and ongoing cochlear pathology such as Meniere's disease (Durrant, 2001; Gravel et al., 2000).

ABRs are objective electrophysiological responses of the brain evoked by auditory stimuli (Stapells, 2002). ABRs are evoked by either clicks or tone-bursts and provide diagnostic information regarding the integrity of the auditory nerve from the cochlea to the lower brainstem. Clinically, click-evoked ABRs are used for the diagnosis of otoneurologic pathologies such as Auditory Dyssynchrony/Neuropathy, Acoustic Neuromas/Vestibular Schwannomas, demyelination such as Multiple Sclerosis, and in newborn infant hearing screenings (Don & Kwong, 2002). Tone-burst ABRs are used to estimate hearing thresholds of infants and difficult to test patients for diagnostic and management purposes (Sininger & Cone-Wesson, 2002).

ABRs and OAEs can be affected by both internal and external factors. Some internal factors include body temperature and internal noise of the individual, whereas some external factors include environmental noise, probe placement, and equipment parameters.

The effects of body temperature on OAEs and ABRs have been studied in both animals and humans. Temperature has been shown to affect frequency, amplitude, and latency of the OAE and the ABR (Khvoles, Freeman & Sohmer, 1998; Taschenberger & Manley, 1997). This paper will first discuss the fundamentals of otoacoustic emissions and auditory brainstem responses and their uses followed by temperature effects on OAEs and ABRs in animals and humans. Lastly, ways to regulate body temperature in the laboratory and the location of body temperature measurement will be discussed. This study will focus on the internal factor of body temperature. It will provide more

information on the effects of changes in body temperature on Distortion Product Otoacoustic Emissions and tone-burst Auditory Brainstem Reponses in the pigmented rat.

CHAPTER 2

LITERATURE REVIEW

A Brief History of Otoacoustic Emissions

In 1928, von Bekesy discovered that the basilar membrane moves in response to sound, with a traveling wave (as cited in Kemp, 2002). A traveling wave is an up and down motion of the basilar membrane which moves from the base to the apex of the cochlea with the point of maximum vibration corresponding to the frequency of the incoming sound. Although the pioneering work of von Bekesy resulted in a Nobel Prize in medicine in the 1950s, his model of cochlear vibration was incomplete. Von Bekesy worked with cadaver ears and inanimate models, which were devoid of any active mechanisms. However, von Bekesy's theory could not adequately explain how many frequencies the human ear can detect and the exquisite pitch perception demonstrated by listeners. This exquisite pitch perception is also known as the ear's ability for "sharp tuning" of sound. Gold (1948) described the theoretical basis of an obligatory physiological phenomenon in the ear to improve and sharpen the responses of the cochlea and suggested an "electromechanical action takes place whereby a supply of electrical energy is employed to counteract the damping." In 1971, Rhode conducted a study on living squirrels and found better frequency resolution than what von Bekesy described with the traveling wave, thus showing that the "sharp tuning" is an active mechanism that occurs in a living cochlea rather than in cadavers or inanimate models used by von Bekesy. A few years later, Kemp (1978) discovered that by inserting a probe into the

ear, sound energy could be detected and measured in the ear canal. Brownell (1983) observed the length of the OHC changing due to changes in voltage, which led to linking the responsibility of the OHCs to the "sharp tuning" of the cochlea. This motion enhances the sensitivity of the cochlea for low-intensity sounds (Neely & Kim, 1983). The motility of the outer hair cells, first described by Davis (1983), is the active movement of the outer hair cells and is commonly called the "cochlear amplifier" (Davis, 1983; Seifert et al., 2001). As a result of the theories and discovery of OHC movement, the sound energy or "echoes" detected in the ear are called otoacoustic emissions (OAEs) and are widely used clinically today. A study conducted by Gorga (1997) provides clinically applicable information regarding Distortion Product Otoacoustic Emissions (DPOAE). Due to this study, DPOAE results can be plotted on a DP-Gram and compared to normative data for clinical purposes.

Otoacoustic Emissions

The human ear is an amazing organ. It almost instantaneously converts acoustic energy into neural impulses for transport through the nervous system. The acoustic energy from the environment enters the external auditory canal and reaches the tympanic membrane, where it is converted into mechanical energy. The mechanical energy moves through the middle ear and continues into the cochlea via the in and out motion of the stapes at the oval window. This motion creates the traveling wave motion of the basilar membrane, which is translated into electro-chemical energy. The energy travels from the hair cells via electro-chemical impulses through the auditory nerve via the brainstem and diencephalon to the cortex for perception of sound.

The transduction between the mechanical motion of the basilar membrane into the electro-chemical signal sent down the auditory nerve begins in the sensory organ of hearing, the Organ of Corti. More specifically, the starting point of this transduction is at the ion channels at the superior portion of the hair cell stereocilia. When stereocilia bend in response to the shearing force created by the traveling wave, potassium ions enter the channels (depolarization). If the intensity of the depolarization is strong enough due to the release of sufficient neurotransmitters, it results in a compound action potential (CAP) at the auditory nerve. The CAP consists of electro-chemical impulses from a group of nerve fibers. This collective impulse that begins at the auditory nerve, continues through the central auditory nervous system (CANS) to the brain, where it is interpreted as sound.

The Organ of Corti consists of a myriad of cells including inner hair cells (IHC), outer hair cells (OHC) and various supporting cells. The IHCs and OHCs have quite a different function in the auditory process. The OHCs function as motors that amplify the incoming sound and the IHCs act as sensory receptor cells that transduce the motion of the traveling wave into electro-chemical impulses. Approximately 95% of the afferent neurons of the auditory portion of the VIII nerve innervate the IHCs, whereas, only about 5% project to the OHCs (Spoendlin, 1969). The outer hair cell motor function or motility observed by Brownell (1984) enhances the sensitivity of the cochlea for low-intensity sounds (Neely & Kim, 1983). The motility of the outer hair cells, first described by Davis (1983), is the active movement of the outer hair cells and is commonly called the "cochlear amplifier" (Davis, 1983; Seifert et al., 2001) because the motion amplifies the magnitude of the signal at the basilar membrane. The amplification process is non-linear,

meaning there is greater amplification from low compared to high intensity sounds (Davis, 1983). The motion of the OHCs is transmitted in reverse of the normal sound transmission path, that is, the movement of the OHCs is sent from the apex to the base of the cochlea, through the middle ear from stapes to malleus, and to the outer ear via the tympanic membrane motion, which acts like the cone from a loudspeaker. The motion of the tympanic membrane creates the sounds ("echoes") that can be measured via sensitive microphones and signal averaging techniques (Kemp, 1989 & 1998). This "echo" is known as an otoacoustic emission (OAE). Damage to the OHC system is associated with a hearing loss of up to 60 dB HL and OAEs in these cases are either absent or reduced in amplitude (Gorga et al., 2000).

OAEs provide a way to look at the cochlea, the hearing sensory organ, in isolation given that the middle ear system is free from pathology (Durrant, 2001). In other words, if the middle ear is free from pathology, the OAEs provide a tool for examining the cochlea. However, if middle ear pathology is present, the OAEs may not provide a mechanism to examine the cochlea, because OAEs may be absent due to obstruction in the forward and reverse transmission of the signal. The dynamic range of OAEs is approximately 30 dB HL. This means that in most cases, TEOAEs will not be present in humans with a hearing status worse than 30 dB HL (mild to moderate hearing loss) and DPOAEs will be reduced in amplitude with greater degrees of hearing loss (Durrant, 2001; Sininger & Abdala, 1998). Studies have shown that a decrease in OAE amplitude is correlated with dysfunction of outer hair cells (Durrant, 2001; Gorga et al., 2000). Clinically, OAEs are a useful way to determine whether the OHCs are functioning properly.

There are two general categories of Otoacoustic Emissions (OAEs): evoked and spontaneous. Evoked OAEs result from a stimulus entering the ear and are generally evoked either by clicks or tones for diagnostic purposes. OAEs evoked by clicks are called Transient Evoked Otoacoustic Emissions (TEOAEs) and OAEs evoked by tones are called Distortion Product Otoacoustic Emissions (DPOAEs). Spontaneous Otoacoustic Emissions (SOAEs) occur in approximately 50% of normally functioning ears without the presentation of an external sound (Prieve & Fitzgerald, 2002). SOAEs occur between 500 Hz and 3000 Hz and commonly range in amplitude from –10 dB SPL to 8.5 dB SPL (Prieve & Fitzgerald, 2002; Sininger & Abdala, 1998). The amplitude of SOAEs is typically higher in neonates than in adults and is more prevalent in females than in males (Sininger & Abdala, 1998). DPOAEs use pure tone stimuli; therefore, DPOAEs are targeting a more narrow and predictable region of the basilar membrane allowing for more frequency specific information. TEOAEs use click stimuli; therefore they stimulate a broad region of the basilar membrane (Kemp, 1998).

DPOAEs are evoked by presenting two tones close in frequency into the ear. The two tones interact with each other and cause additional vibrations (other than the two primary frequencies presented) along the basilar membrane. These vibrations cause a distortion or an intermodulation vibration demonstrating the non-linearity of the cochlea. The distorted echo (distortion product) is best seen at 2f1-f2, where f1 is the lower frequency and f2 is the higher frequency. The f2/f1 presentation ratio is typically 1.22 because it has been shown to result in the largest OAE (Gorga et al., 2000; Kemp, 1998).

OAEs are measured by inserting a probe with a microphone into the external ear canal. The microphone measures the otoacoustic emission and relays the information to

a computer for signal averaging. The results are displayed in a graphic or tabular form on the computer screen. In order to interpret the OAE results, a clinician should look at several parameters including noise, reproducibility, the temporal waveform, response amplitude, and signal to noise ratio of the emission. Robust DPOAE responses across the entire frequency range indicate that OHC structures in the cochlea needed for normal hearing are intact and functioning normally.

This study focuses on distortion product otoacoustic emissions because DPOAEs provide frequency specific information at the level of the OHCs of the cochlea, in order to help predict hearing sensitivity thresholds. Due to the fact that studies have shown higher referral rates for diagnostic testing when using only OAEs as the screening tool in newborns, Auditory Brainstem Response will also be discussed. High referral rates could be, in part, due to not taking body temperature into account and this issue will also be explored further in this paper (Clarke, Iqbal, & Mitchell, 2003; Lemons et al., 2002; Vohr et al., 2001).

Auditory Brainstem Response

After sound has traveled through the above-mentioned pathway, at the location of the IHCs the energy is transmitted to the auditory nerve and then travels to the auditory cortex for the perception of sound. The distal portion of the auditory nerve is the location where the earliest component of the ABR, wave I, is generated. ABRs can be evoked either by clicks or tone-bursts for diagnostic purposes. Clicks are used for otoneurologic diagnostics such as Auditory Dyssynchrony/Neuropathy, Acoustic Neuromas/Vestibular Schwannomas, demyelination such as Multiple Sclerosis, and in newborn infant hearing screenings (Don & Kwong, 2002). When looking at a click-evoked ABR waveform to a

high intensity click stimulus, it consists of five main peaks labeled as waves I, II, III, IV, and V. The main components of interest in the click-evoked waveform are the amplitudes and latencies of waves I, III, and V, interpeak latencies of I-III, III-V and I-V, interaural amplitude and latency (IT5) comparisons of wave V, the ratio of V/I amplitude, and interaural comparison of V/I ratio (Don & Kwong, 2002).

Tone-burst ABRs are used to estimate hearing thresholds of infants and difficult to test patients. The tone-burst ABR primarily consists of wave V followed by the negative component following wave V, known as V' (Stapells, Gravel, & Martin, 1995). Wave V has a larger amplitude at suprathreshold intensities, and as the intensity approaches the hearing threshold the amplitude diminishes. The lowest intensity at which the presence of wave V is repeatable is considered the hearing threshold of the patient. Stapells et al. (1995), found that more than 90% of normal-hearing infants had ABR responses to 30 dB nHL for 500 Hz tones and between 96-100% had responses to 20 dB nHL for 2000 and 4000 Hz tones.

To record ABRs, a common electrode scheme, called a montage, is to use four electrodes placed on the patient's head and deliver either a click or a tone-burst to the ear via an insert earphone. The four electrode locations are A1 and A2, Cz, and Fpz. A1 and A2 are the negative or inverting, reference electrodes located on the earlobes. Cz is the positive or non-inverting, active electrode placed on the crown of the head, half way between the pre-auricular region of each ear and half way between the inion and nasion. Fpz is the ground electrode placed on the forehead. This study will focus on tone-burst ABRs as opposed to click ABRs. To date, there have been a limited number of studies on either animals or humans using tone-burst ABR results compared to OAE results.

Uses of Otoacoustic Emissions and Auditory Brainstem Responses

OAEs were used in many clinic and research settings once a commercially OAE diagnostic system was available in 1988 (ILO88). In the clinic, OAEs can be used as part of a basic test battery to help confirm pure-tone behavioral results. They can be particularly helpful in workman's compensation cases to help detect malingering. Additional uses of measuring OAEs are monitoring of ototoxicity, occupational noise exposure, and diagnosis and management of Meniere's disease (Durrant, 2001; Gravel et al., 2000). The most popular and commonly discussed application of otoacoustic emission testing in audiology is its use in newborn hearing screenings. OAE measurement is quick, simple, and non-invasive which is especially useful when testing infants (Ferber-Viart et al., 1995; Gorga et al., 2000; Taschenberger & Manley, 1997).

As previously discussed, click-evoked ABRs are used for otoneurologic diagnostics such as Auditory Dyssynchrony/Neuropathy, Acoustic Neuromas/Vestibular Schwannomas, demyelination such as Multiple Sclerosis, and in newborn infant hearing screenings and tone-burst ABRs are used to estimate hearing thresholds of infants and difficult to test patients (Don & Kwong, 2002; Sininger & Cone-Wesson, 2002). In 1971, the first commercial ABR equipment for clinical use, known as the ERA 70, was made available. Eight years later, Davis coined the term "ABR" (GN Otometrics). Today's technology provides us with an automated ABR (AABR). The commercially available AABR screener is an ALGO-1. The battery operated ALGO-1 uses a 35 dB nHL alternating click stimulus presented at a rate of 37 pulses per second. The stimulus is presented monaurally, and then results are predicted on a statistical likelihood ratio

model. If the outcome meets the criteria, "pass" is displayed on the screen and if not, then "refer" is displayed (Jacobson, J. T., Jacobson, C. A., & Spahr, 1990).

Universal Newborn Hearing Screening (UNHS) requires an objective measure of auditory status in patients who cannot provide a reliable behavioral response. There are two common methods used to objectively measure auditory status: Auditory Brainstem Response (ABR) and Otoacoustic Emissions (OAEs).

ABRs and OAEs both have advantages and disadvantages. ABRs can provide diagnostic information regarding portions of the auditory system medial to the cochlea and in the lower portions of the brainstem. ABRs are less affected by the middle ear status than OAEs (Cacace & Pinheiro, 2002). Both ABRs and OAEs are noninvasive, however the neonate can be awake while OAE results are obtained. In order to obtain an ABR, the neonate must be asleep (NIH, 1993; Stone, Smith, Lembke, Clarke, & McLellan, 2000). Due to the use of electrodes, the setup time for ABRs takes a bit longer than for OAEs (Meier, Narabayashi, Probst, & Schmuziger, 2004; NIH, 1993). OAEs primarily provide information about the functioning of the OHCs, however they are dependent on the status of the middle ear, and can be difficult to detect in the presence of intrusive background noise (Cacace & Pinheiro, 2002).

With today's technology, after taking the total screening time, cost, and referral rates into account, AABRs are comparable to and in some aspects better than using OAE an a screener. The evidence to support this claim is based on several factors. First, the total time for an AABR screening range from 45 seconds to 25 minutes depending on the equipment being used (Clarke et al., 2003; Lemons et al., 2002; Jacobson et al., 1990; Meier et al., 2004; Meyer et al., 1999). Similarly, the total time for an OAE screening

ranges from 30 seconds to 20 minutes (Clarke et al., 2003; Meier et al., 2004; Lemons et al., 2002; Stone et al. 2000). Secondly, several studies have shown that the referral rates for AABRs are generally lower than referral rates for OAEs. The referral rates for AABRs range from 3.21% to 6% while the referral rates for OAEs range from 6.49% to 33.3% (Clarke et al., 2003; Lemons et al., 2002; Vohr et al., 2001). A study conducted by Jacobson et al. (1990) determined the sensitivity and specificity of initial screening results of the ALGO-1 to be 89% and 96%, respectively, with sensitivity and specificity of retest analysis as 100% and 96% respectively.

In March 1993, the National Institutes of Health (NIH) Consensus Statement recommended that "the preferred model for screening should begin with an evoked otoacoustic emissions test and should be followed by an auditory brainstem response test for all infants who fail the evoked otoacoustic emissions test." Acceptable screening protocols recommended by the National Institute on Deafness and Other Communication Disorders (1997) are: 1) ABR, 2) Transient Evoked Otoacoustic Emissions (TEOAEs) or Distortion Product Otoacoustic Emissions (DPOAEs), or 3) a combination of both OAE and ABR. A study by Gravel et al. (2000) looked at newborn hearing screening protocols in several hospitals and showed that by using both the TEOAE and ABR procedures the false-positive rates were significantly lowered compared with using either technique alone. Overall, using the OAE and ABR combination results in only slightly higher refer rates (8.6%) and the cost is only a bit higher than using OAE or ABR alone (Clarke et al., 2003; Vohr et al., 2001).

There are many possible reasons for high false-positive rates. They may partially be due to the fact that testing was incorrectly conducted. High false-positive rates are

undesirable because they not only cause unnecessary anxiety to the parents; but they also add additional costs to the diagnosis by sending the infant for retesting. Therefore, it is important to minimize the number of false positives. Since cost is a concern when conducting hearing screenings, Gravel et al. (2000) stated that the NICU is in greater need of the screening compared with well-baby nurseries because a higher prevalence of hearing loss occurs in the NICU. Mencher, Davis, DeVoe, and Beresford (2000) agreed that the NICU should have priority over the well-baby nursery especially when resources are limited. A study conducted in the Philippines by Chiong, Llanes, Tirona-Remulla, Calaquian, and Reyes-Quintos (2003) involving a large number of infants (n=435) looked at pass and referral rates of children tested with DPOAEs and the reasons for the referrals. The referral rate in this study was 49.2% of all infants screened. The authors claimed this referral rate is similar to the referral rate of a "parallel study conducted locally" (no citation given), but considerably higher than the referral rates in other studies: 6.63 % (Spivak et al. 2000) and 5.3% (Meyer et al. 1999). According to Chiong et al., this uncited work re-screened 12 of the referrals. Nine, or 75% of the 12 infants rescreened passed.

In order to help decrease the number of false-positive referrals, Gorga et al. (2000) suggested that the optimal time to conduct the screening is just before discharge when there are few factors contributing to the screening results such as the infants' health and vernix. According to Clarke et al. (2003), when neonates are screened more than 23 hours postnatal there are fewer referrals. In addition to the infant's health and exterior ear canal condition, Gehr, Janssen, Michaelis, Deingruber, and Lamm (2004) stated that the middle ear status is an important aspect to monitor during a newborn hearing

screening. The study by Chiong et al. (2003) mentioned that high referral rates in the NICU may be due to the fact that testing was performed on high-risk neonates or due to background noise in the testing environment. Meyer et al. (1999) suggested that OAEs should not be used for screening high-risk neonates. Chiong et al. assessed risk factors including gender, age, Apgar scores, birth weight, perinatal history, caesarian section, neonatal diseases, medication, mother's age, bleeding, pre-eclamsia, and mother's diseases. Of these, the only significant finding ($p=0.012$) for the referral response was the male gender, which is not a justifiable factor that could affect the referral rate. In addition, Chiong et al. did not discuss other factors, particularly, temperature of the infants that may need to be taken into account.

Effects of Temperature on OAEs and ABRs

In order to optimize testing procedures, it is important to understand the relationship between the stimulus and endogenous traits of individuals when conducting tests of the auditory system (Cacace, McClelland, Weiner, & McFarland, 1996). Eisenberg (1976) discussed the importance of temperature, specifically room temperature and its overall effects on infants. Infants become lethargic and respond less to external stimuli when the room temperature is below approximately 79°F. Above 81°F infants become irritable and it becomes difficult to detect behavioral responses to sound from the infant due to the infant's movements (Eisenberg, 1976). Studies have found that a variety of non-auditory physical attributes can affect OAEs. For example, one study by Cacace et al. looked at time of day, resting pulse rate, gender, sound pressure levels (SPL), and stimulus frequency on the amplitude of DPOAEs. The OAE amplitude was not affected by time of day and resting pulse rate; however, the amplitude was

significantly different between males and females (females had larger DPOAEs than males), SPL levels, and stimulus frequency were significant. Studies have also investigated the effects of body temperature on ABRs and OAEs.

Hypothermic (body temperatures below that of normal body temperature) and hyperthermic (body temperatures above that of normal body temperature) body temperature changes have been shown to affect the results of ABRs and OAEs in both humans and animals (Ferber-Viart et al., 1995; Gold, Cahani, Sohmer, Horowitz, & Shahar, 1985; Janssen, Hetzler, Creason, & Dyer, 1991; Khvoles et al., 1998; Markland et al., 1987; Nielsen & Jessen, 1992; O'Brien, 1994; Seifert et al., 1998; Seifert et al., 2001; Starr et al., 1998; Taschenberger & Manley; 1997). Seifert et al. (1998) stated that the effects of temperature in amphibians are well known; however, due to the physical differences in the cochlea, such as amphibians having no basilar membrane or apparent difference between IHCs and OHCs between amphibians and humans, it makes it difficult to compare amphibian cochlear function to that of humans. Studies conducted on animals, however, provide insight into the general effect of temperature on otoacoustic emissions.

The following sections will provide discussions into some ways body temperature can be changed and the best location of measuring body temperature for research purposes.

Means of regulating body temperature.

In studies conducted on both animals and humans, body temperature has been regulated in several ways including by a thermal blanket, climatic chamber, water bath, fever, heating pad, exercise and surgery. A study by Neilsen and Jessen (1992) had their

human subjects wear long pants and long-sleeved shirts while exercising for thirty to forty minutes to raise their body temperature approximately 4°C. Taschenberger and Manley (1997) used a thermal blanket to change the body temperature of barn owls from 1°C below to 3°C above normal body temperature. A study done by Ferber-Viart et al. (1995) used a climatic chamber, with a monitoring system to regulate both the humidity and temperature. Another study by O'Brien (1994) measured the body's natural temperature changes due to a fever caused by a urinary tract infection. Lastly, a study by Seifert et al. (1998) observed OAEs while monitoring a reduction in body temperature during heart surgery. A reduction of body temperature is a standard protocol used during heart surgery to reduce the amount of blood flow.

Location of body temperature measurement.

In addition to various regulation methods, temperature has been measured in various places. Some locations of measurement include oral, rectal, non-test ear, near the tympanic membrane, urinary bladder, and nasopharynx. O'Brien (1994) used a thermometer that measured infrared radiation from the tympanic membrane. For comparison purposes, Seifert et al. (1998) measured temperature in both the nasopharyngeal cavity as well as the urinary bladder. Seifert and colleagues (1998) reported that the nasopharynx seems to be the most sensitive and accurate way to measure temperature changes in the cochlea due to its close anatomical proximity to the fluid in the cochlea. Taschenberger and Manley (1997) rectally measured the body temperature of the barn owl. Cacace et al. (1996) measured oral temperature and resting pulse rate; however, there was no significant relation among these two.

Effects of temperature on OAEs in animals.

Martin, Hudspeth, and Jülicher (2001) conducted a study that attempted to determine whether the spontaneous movements of the stereocilia on hair cells in the bullfrog were truly active or if they were based on fluctuations caused by temperature changes. This study used an elaborate equation called the fluctuation-dissipation theorem (FDT). The FDT "provides a useful instance of such a principle that assumes no physical properties of the system under investigation other than thermal equilibrium" (Martin et al, 2001). Using this equation, the investigation demonstrated that spontaneous movements of stereocilia were based on temperature changes.

A study done by Taschenberger and Manley (1997) measured whether SOAEs were affected by temperature changes in the barn owl. One benefit to studying barn owls is their "many-to-one" innervation pattern of afferent fibers is similar to that of the IHCs of humans, which makes it easier to generalize findings to humans (Taschenberger & Manley, 1997). In order to control for any changes in head position, a metal rod was cemented to the barn owls' skulls while under anesthesia. To ensure that any changes in SOAE amplitude were due to temperature, this study accounted for the natural small fluctuations in amplitude by monitoring suppression effects. Taschenberger and Manley (1997) found that as temperature was raised, the SOAE shifted to a higher frequency. Conversely, as temperature was lowered, the SOAE was shifted to a lower frequency. Similarly, Long, Van Dijk, and Wit (1996) reported that as temperature decreased, the frequency of the SOAEs decreased in frogs.

Seifert et al. (2001) studied the effects of temperature on the amplitude of TEOAEs in 21 guinea pigs. This study demonstrated that as temperature decreased, the

amplitude of the TEOAE also decreased. A study by Khvoles et al. (1998) showed no significant reduction in TEOAE or DPOAE amplitude when cooling a rat to 33°C. However, cooling the body temperature from 33°C to 27°C did produce a reduction in amplitude of both TEOAEs and DPOAEs. Similarly, a decrease in TEOAE and DPOAE amplitude was also present when body temperature was raised to 39°C and 40°C (Khvoles et al., 1998). These findings suggest that the changes in OAE amplitude are affected by the amount the animals change in body temperature. Death occurred in rats at high body temperatures of 41°C and 42°C (Khvoles et al., 1998).

Effect of temperature on OAEs in humans.

In addition to temperature affecting OAEs in animals, it has also been shown to have effects on OAEs in humans. O'Brien (1994) studied the SOAEs in one male with a urinary tract infection. He found that as the body temperature increased, both the amplitude and the frequency of the SOAE decreased. A study done by Cacace et al. (1996) looked at the DPOAEs in 16 normal hearing humans. Their findings suggested a significant main effect of time of day for oral temperature.

Ferber-Viart et al. (1995) was the first study to examine the effects of hyperthermia on TEOAEs in humans. During the TEOAE measurements, the body temperature for this sample of young men (n=7) ranged from 36.6°C to 38.5°C. At a room temperature of 25.0°C, the subjects' body temperature was 36.6°C to 37.3°C. When the room temperature was increased to 40.0°C, the subjects' body temperature reached 38.5°C. TEOAEs were monitored as body temperature was increased and then lowered back to normal body temperature. Generally, their results showed that an increase in body temperature caused the amplitude of the TEOAE to decrease. After the

room temperature reached a temperature of 40.0°C and the measurements were made, the room temperature was then decreased back down to 25.0°C. For this study, the only statistically significant finding was an increase in amplitude of the TEOAE during the cooling process of the room back to 25.0°C. However, a study conducted by Starr et al. (1998) on a six-year old girl with a fever of 38.1°C showed no change in TEOAE amplitude.

Effects of hypothermia on OAEs in humans have also been observed. Seifert et al. (1998) observed TEOAEs in humans ranging from infants to the elderly during heart surgery in which the body is cooled as part of the surgery protocol. He took these temperature readings using two approaches: 1) nasopharyngeal and 2) vesical (urinary bladder). He reported that as the body temperature of the individual decreased, the amplitude of the TEOAE also decreased. During the cooling process, the temperature at which the TEOAE disappeared was higher than the temperature when the TEOAE reappeared during the re-warming process. The average difference between when the TEOAE disappeared and when it reappeared was 2.67°C for the nasopharyngeal measurement and 4.66°C for the vesical measurement. On average, the nasopharyngeal temperature was 3.25°C lower than the vesical temperature during cooling, only 1.21°C lower during the steady state of hypothermia, and 0.05°C lower during re-warming. Seifert et al. stated that although there is a lag between nasopharyngeal or vesical temperature and their target organs (brain and cochlea), this study shows clear influences of temperature on TEOAEs.

Effects of temperature on ABRs in animals.

A limited number of ABR studies have been conducted on rats in both the hypothermic and hyperthermic state (Gold et al., 1985; Janssen et al., 1991; Khvoles et al., 1998).

Results of the hyperthermic study by Gold et al. (1985) indicated that heating caused a decrease in latency of the ABR waves. The greatest change in latency occurred in wave IV. For example, at a body temperature of 37°C, the absolute latency of wave IV was 3.7 ms and when heated to 42°C, the absolute latency was 3.0 ms; a shift of 0.7 ms, whereas at the same body temperatures, wave I had a latency shift of only 0.1 ms (Gold et al., 1985). In addition to latency, the amplitude of ABR waves I, III, and IV also decreased (Gold et al., 1985).

Hypothermic ABR studies demonstrated that a decrease in body temperature from 37.5°C to 36.5°C (Janssen et al., 1991) and 37°C to 27°C (Khvoles et al., 1998) resulted in an increase in ABR latency. On average, the latency of wave I increased from approximately 1.497 ms to 1.518 ms and wave IV from 3.37 ms to 3.85 ms in the study by Janssen et al., similarly, wave I was approximately 0.55 ms longer at 27°C than at 37°C in the study conducted by Khvoles et al. In addition, the air-conducted ABR thresholds were elevated by 25 dB at 27°C in comparison to the air-conducted ABR thresholds obtained at 37°C (Khvoles et al., 1998). In addition to latency, the study conducted by Janssen et al. examined amplitude and showed a significant increase ($p < 0.0016$) in the amplitude of all ABR waves as the temperature decreased.

Effects of temperature on ABRs in humans.

Studies investigating the effects of both hypothermic and hyperthermic body temperatures on click-evoked ABRs have been conducted on humans (Markland et al., 1987; Nielsen & Jessen, 1992; Starr et al., 1998). In hyperthermic conditions, Nielsen and Jessen (1992) found that when body temperature is raised approximately 4°C; the ABR results produce a shortened interpeak latency of waves I-V, I-III, and III-V. Starr et al. (1998) tested a six-year old girl with auditory neuropathy when a fever was present and without a fever. When the girl had a fever, Starr et al. (1998) found that the ABR was absent and when she did not have a fever, wave V was present but delayed in latency.

In a hypothermic study by Markland et al. (1987), when body temperatures were lowered between 36°C and 20°C, a latency shift occurred in all the ABR waves. The colder temperatures caused longer absolute latencies for each wave. These findings are consistent with hypothermic ABR studies on rats (Janssen et al., 1991; Khvoles et al., 1998). Markland et al. (1987) also showed that interpeak latencies of I-III, III-V, and I-V increased as body temperature decreased. According to Markland et al. (1987), for every 1°C decrease in temperature, there is approximately a 7% increase in the interpeak latency of I-V. Several theories exist as to what causes latency shifts to occur due to changes in temperature.

Temperature Trends

In both animals and humans, otoacoustic emission amplitudes generally tend to decrease when body temperature changes, whether raised or lowered, from normal body

temperature (Ferber-Viart et al., 1995; Markland et al., 1987; O'Brien, 1994; Seifert et al., 2001; Seifert et al., 1998; Taschenberger, & Manley, 1997). The most common speculation for this change is the slowing of conduction along the axon of the nerve as well as the slowing of synaptic transmission (Markland et al., 1987). O'Brien (1994) suggested that changes in body temperature change the physical properties of the fluids in the cochlea, which consequently will result in OAE differences. Ferber-Viart et al. (1995) mentioned increases in body temperature could cause a vasomotor reaction, "a dilation or constriction of blood vessels", which could cause changes in middle ear pressure and thus cause a reduction in the amplitude of the otoacoustic emissions. These results agree with findings from a study conducted by Seifert et al. (1998) which indicated that a decrease in body temperature can also produce a decrease in middle ear pressure, ultimately resulting in an overall decrease in TEOAE amplitude. The results of the study done by Ferber-Viart et al. (1995) suggest that temperature showed some effect on OHC movement. It is possible that temperature could indirectly change OHC movement by affecting the channel-gating kinetics or the metabolic pathways (Ferber-Viart et al., 1995).

Taschenberger and Manley (1997) reported that when body temperature increased, the frequency of the SOAE shifted to a higher frequency. On average, the frequency of the SOAE shifted by a factor of 0.039 octaves per one degree Celsius change in body temperature from normal body temperature. Their results also appear to indicate the further the shift in body temperature from normal temperature, the greater the shift in the frequency of the SOAE. The largest frequency shift in SOAE occurred at 531 Hz and changed 0.084 octave/°C.

Cacace et al. (1996) found a significant main effect for time of day on oral temperature; however, they also claimed that changes in oral temperature were not significantly correlated with the DPOAE changes in a 24-hour time period, which is consistent with other studies that showed little temperature dependence (Whitehead, Wilson, & Baker, 1986; Wilson, 1985).

Statement of the Problem

Studies have shown that even small changes in body temperature may cause changes in otoacoustic emission amplitude as well as changes in the amplitude and latency of the auditory brainstem response. These changes have primarily been explored in animals with a few studies on humans. The current study requires the use of animals due to extreme temperature changes. To date, few studies have explored the effects of temperature on tone-burst ABRs and DPOAEs. Finally, little is known as to whether or not these temperature effects on OAEs occur at very high frequencies up to 16 kHz, because the highest frequency tested in the OAE literature is 8 kHz (Khvoles et al., 1998). This study will explore temperature effects of DPOAEs on frequencies up to 16,000 Hz in rats. The dynamic range of hearing in rats is from 250 Hz to 80,000 Hz (Crofton, Lassiter, & Rebert, 1994).

Two additional questions that remain unanswered are: Should taking body temperature before performing OAEs and ABRs be part of the standard test protocol? And, should normative data be collected based on body temperature?

Therefore, this study was designed to provide more information on the effects of body temperature on Distortion Product Otoacoustic Emissions and tone-burst Auditory Brainstem Reponses in the pigmented rat.

CHAPTER 3

METHODOLOGY

Sixteen pigmented rats were used as subjects for this study. The average weight of these rats was 388g and they were not used in any previous study. This protocol was submitted to the Institutional Animal Care and Use Committee (IACUC) at the University of Maryland Medical School and the approval is included as Appendix A. Distortion Product Otoacoustic Emissions (DPOAEs) and Auditory Brainstem Response (ABR) were assessed during various body temperatures as discussed below.

Otoacoustic emissions

A Starkey Labs DP 2000 DPOAE Measurement System was used to record the DPOAEs in the rats. An insert earphone was used to deliver the stimulus to the left ear of each rat. A DPOAE was considered acceptable when the DPOAE amplitude was at least 3 dB above the noise level. $2f_1$-f_2 DPOAEs were measured at 2k-16k Hz using L_1/L_2 intensities of 45/35, 55/45, and 65/55 dB SPL with f_2/f_1 ratio of 1.20. The DPOAEs were measured at six points per octave, repeated twice.

Auditory brainstem response

The BioSig Version 2.0 equipment was used to record tone-burst air conducted frequency-specific ABRs. An insert earphone in the rat's left ear was used to deliver the air-conducted stimulus. Subdermal recording electrodes were placed at the vertex (between the eyes) and just below the left ear with the ground electrode located in the front right limb. A one-channel ABR recording was measured at 3.15k, 6.3k, 10k, 12.5k,

and 20k Hz. A linear-gated tone was presented at a rate of 21.0/s and the ABR was recorded to 1008 sweeps. The temporal waveform was displayed in a 10 ms post-stimulus analysis window. The initial intensity of the stimulus was 30 dB SPL and was decreased in 5 dB SPL steps until threshold was reached. A threshold was determined as the lowest intensity that a repeatable wave V of the ABR was elicited.

Rat preparation

Each rat was anesthetized with Nembutal using 50 mg/kg. After 45 minutes, if the animals were still not sufficiently anesthetized, an additional dosage of Nembutal (between approximately 1/3 and 1/2 of the original dose) was given. Thereafter, 1/3 of the original dose was given every 45 minutes, if needed, judged by the paw withdrawal reflex, until all testing measurements had been completed. All measurements were made with the anesthetized rat placed in a soundproof test booth.

Temperature protocol

In the sixteen rats, body temperature was changed and measurements were made at 37.5º C, 38.5º C, 39.5º C and 40.5º C. DPOAEs and ABRs were measured at all four temperature points. The sixteen rats were randomly divided into two trial groups. The body temperature of the rats in the first trial group (Trial Group 1) was systematically *increased* from a normal body temperature of 37.5º C to 38.5º C, 39.5º C and 40.5º C. The body temperature of the rats in the second trial group (Trial Group 2) were *increased* from a normal body temperature of 37.5º C to 40.5º C, and then systematically *decreased* to 39.5º C, 38.5º C and 37.5º C while DPOAEs and ABRs were measured at each temperature. For both trial groups, initial measurements were always performed at normal body temperature before starting the temperature change process. The body

temperature of the rat was only allowed to fluctuate +/- 0.2º C from the target temperature and was monitored with the use of a thermometer inserted rectally.

Trial Group 1

The body temperature of each rat was maintained at 37.5º C using the Harvard Homeothermic Blanket Control Unit until DPOAEs and ABRs were recorded. The body temperature of each rat was then increased to 38.5º C, 39.5º C, and 40.5º C using a Davol 6A 125V UND Lab, Inc. heating pad and maintained at that temperature using the Harvard Homeothermic Blanket Control Unit until DPOAEs and ABRs were recorded. The order in which the ABR and the DPOAEs were recorded alternated at each temperature. Specifically, at 37.5º C the ABR was recorded first followed by the DPOAEs, at 38.5º C the DPOAEs were recorded first followed by the ABR, at 39.5º C the ABR was recorded first followed by the DPOAEs, and finally at 40.5º C the DPOAEs were recorded first followed by the ABR.

To help speed up the heating process, the heating pad was used on the medium and high settings to increase the body temperature of each rat. Each change in body temperature took approximately 15 minutes. After all measurements were made, each rat was allowed to cool to the normal body temperature of 37.5º C using the Harvard Homeothermic Blanket Control Unit and closely monitored until the Nembutal wore off. The total amount of testing time on each rat in the first trial group took between four and five hours.

Trial Group 2

The body temperature of each rat was maintained at 37.5º C using the Harvard Homeothermic Blanket Control Unit until DPOAEs and ABRs were recorded. The

heating pad was used on the high setting to increase the body temperature of each rat to 40.5º C and maintained using the Harvard Homeothermic Blanket Control Unit while DPOAEs and ABRs were recorded. Next, the Harvard Homeothermic Blanket Control Unit was set appropriately to achieve cooling to 39.5º C, 38.5º C and finally 37.5º C; DPOAEs and ABRs were repeated at each temperature. The order in which the ABR and the DPOAEs were recorded alternated at each temperature. Specifically, at 37.5º C and 39.5º C the DPOAEs were recorded first followed by the ABR; at 38.5º C and 40.5º C the ABR was recorded first followed by the DPOAEs.

As rats control body temperature by their tail, the tail was used to speed up the cooling process by cooling it with cold water. In order to maintain the target temperatures during the cooling process, the heating pad was used on the high setting in conjunction with the Harvard Homeothermic Blanket Control Unit as needed. Once the 37.5º C was reached and after all measurements were made, the rats were kept at their body temperature of 37.5º C and closely monitored until the Nembutal wore off. The total amount of testing time on each rat in the second trial group took between five and six hours.

Data Analysis

After all data from Trial Groups 1 and 2 were collected, the raw data from the Starkey Labs DP 2000 Distortion Product Otoacoustic Emission (DPOAE) measurement system were imported from a Microsoft Access database into an Excel spreadsheet. In Excel, the DPOAE data from two repetitions for each rat were averaged for all frequencies and then transposed into the format required for importing into SPSS for Windows for analysis. The auditory brainstem response (ABR) data were entered

manually into an Excel spreadsheet and then imported into SPSS for Windows for analysis.

The criterion of the response being at least 3 dB above the noise floor to be accepted into analysis was applied to all DPOAE results. Descriptive and inferential statistics were conducted on all available DPOAE F2 frequencies of 2,015 Hz, 2,250 Hz, 2,531 Hz, 2,812 Hz, 3,187 Hz, 3,562 Hz, 3,984 Hz, 4,500 Hz, 5,062 Hz, 5,672 Hz, 6,328 Hz, 7,125 Hz, 8,016 Hz, 9,000 Hz, 10,078 Hz, 11,297 Hz, 12,703 Hz, 14,250 Hz, and 15,984 Hz and the ABR frequencies of 3150 Hz, 6300 Hz, 10,000 Hz, 12,500 Hz, and 20,000 Hz. The DPOAEs and ABRs at different temperatures were analyzed using analysis of variance (ANOVA) at $p < 0.01$ and regression analysis.

CHAPTER 4

RESULTS

Distortion Product Otoacoustic Emissions

Recall the sixteen rats were randomly divided into two equal groups of eight: trial group 1 (TG1) and trial group 2 (TG2) for preliminary analysis. In the case of TG1, temperature was gradually increased from 37.5ºC (normal body temperature for rats) to 40.5ºC; for TG2, after measuring the response for 37.5ºC; the temperature was increased to 40.5ºC and then gradually decreased to 37.5ºC**.** The measurements were made for temperature changes in 1ºC steps. DPOAEs were tested twice in each condition, and the trials were averaged for use in descriptive and inferential analysis.

The DPOAE amplitude from 2,000 to 16,000 Hz for each temperature is illustrated in Figure 1 for TG1 and Figure 2 for TG2. For both groups, examination of these figures for all four temperatures indicated that the average DPOAE amplitude was between 0 and 10 dB SPL up to about 4,000 Hz, sharply increased from 4,000 to 5,000 Hz and remained between 20 and 30 dB SPL from 5,000 Hz to about 10,000 Hz. At approximately 11,000 Hz, the average amplitude suddenly decreased about 10 dB SPL, and then abruptly increased by 10 dB SPL at the next adjacent frequency (12,700 Hz). This pattern is similar for TG1 and TG2, however note that for TG1 there was slight separation for each temperature at 5,000 Hz and above.

A 4x19 (temperature x frequency) repeated measures analysis of variance was conducted on TG1. There was a significant main effect for temperature $F(4, 28) =$

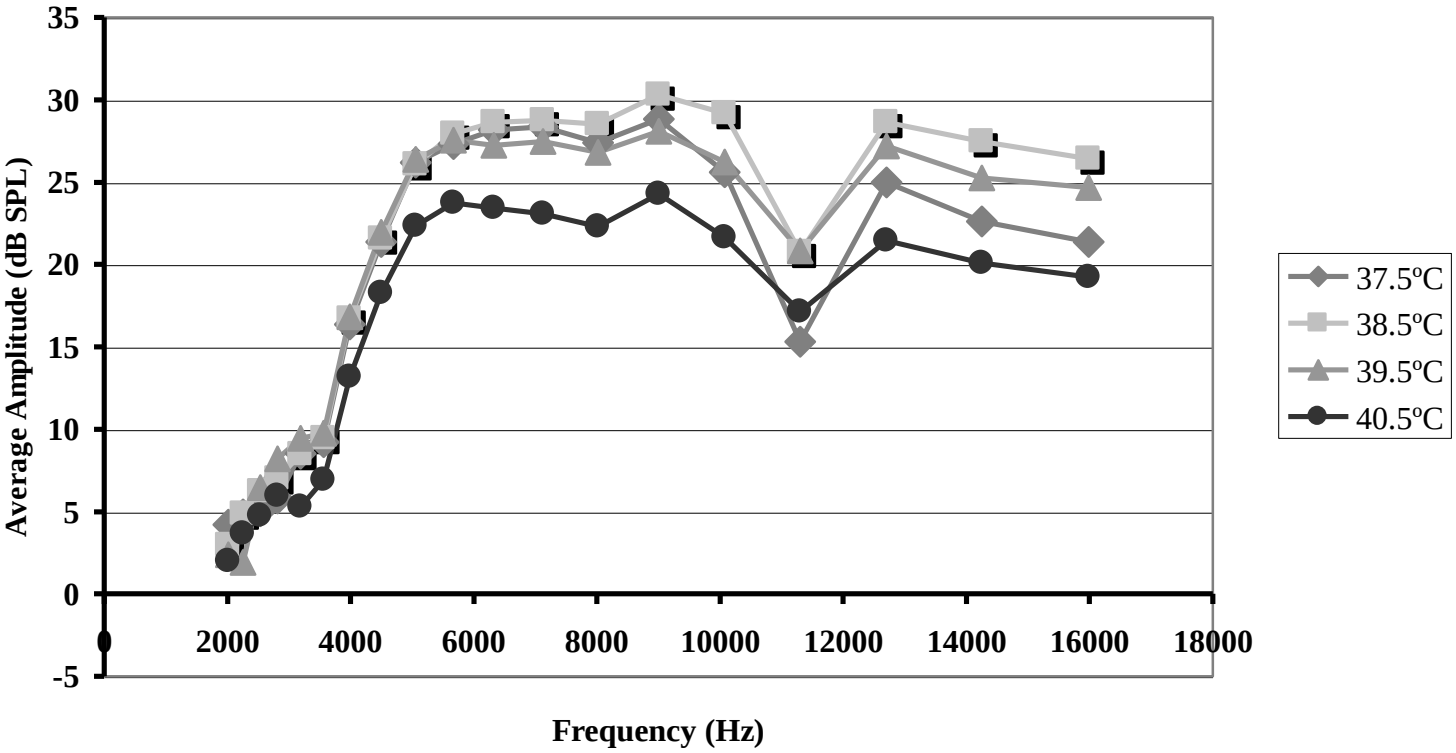

Figure 1. DPOAE results (averages for 8 rats) for measurements performed at temperatures of 37.5ºC, 38.5ºC, 39.5ºC and 40.5ºC for trial group 1.

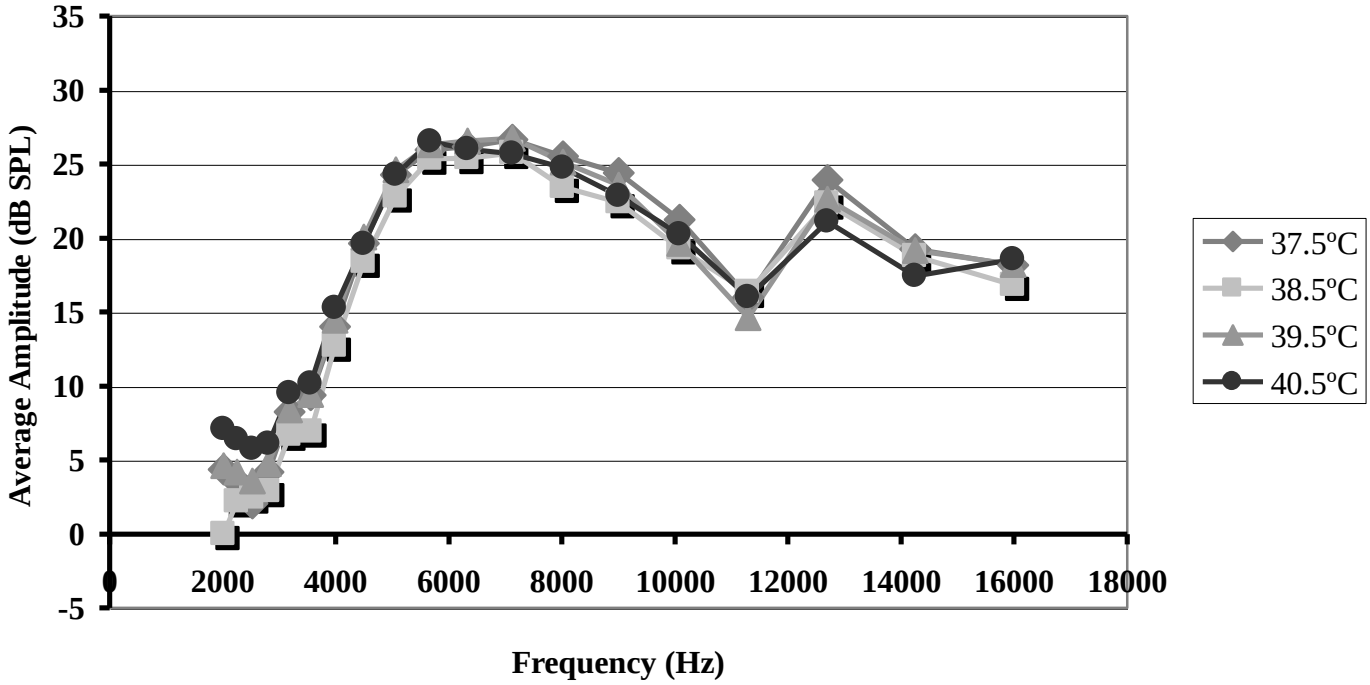

Figure 2. DPOAE results (averages for 8 rats) for measurements performed at temperatures of 37.5ºC, 38.5ºC, 39.5ºC and 40.5ºC for trial group 2.

4.598, $p = 0.006$ and frequency F (18, 126) = 35.598, $p = 0.000$, but no significant interaction between temperature and frequency (Table 1). A 5x19 (temperature x frequency) repeated measures analysis of variance was also conducted for TG2. There was a significant main effect for frequency F (18, 126) = 77.261, $p = 0.000$, but not for temperature or the interaction between temperature and frequency (Table 2).

A 2x19 (trial x frequency) repeated measures analysis of variance was conducted to determine the reliability of the 37.5ºC DPOAE measurements for TG2, when the measurements were taken at the beginning of data collection (initial baseline temperature) and after all other temperatures had been tested (final temperature/returned to baseline temperature). The results of this ANOVA indicated a significant main effect for frequency F (18, 126) = 36.419, $p = 0.000$, but no significant main effect for trial or interaction between trial and frequency (Table 3). The results are presented in Figure 3.

A 2x19 (group x frequency) mixed model analysis of variance was conducted to compare the initial body temperature of 37.5ºC for TG1 to the initial body temperature of 37.5ºC for TG2. The results of this analysis indicated a significant main effect for frequency F (18) = 66.732, $p = 0.000$, but no significant effect for trial group or the interaction between trial group and frequency (Table 4) indicating the groups appeared to be equal at baseline for DPOAE and temperature. The results are presented in Figure 4.

Independent sample *t* tests were conducted at all frequencies to compare the DPOAE measurements obtained for trial group 1 versus trial group 2 at the initial body temperature of 37.5ºC. As shown in Table 5, the results of the independent *t* tests revealed there was no significant difference in DPOAE results for group 1 versus group 2 at any of the test frequencies. Based on a Levene's test of equality of variance,

Table 1.

Tests of Within-Subjects Effects results of ANOVA for DPOAEs on TG1.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
temp	2647.367	4, 28	661.842	4.598	*.006
frq	51504.256	18, 126	2861.348	35.598	*.000
temp * frq	578.538	72, 504	8.035	.686	. 975

Note. temp = temperature; frq = frequency; * $p < 0.01$.

Table 2.

Tests of Within-Subjects Effects results of ANOVA for DPOAEs on TG2.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
temp	1568.940	3, 21	522.980	1.148	. 353
frq	49002.352	18, 126	2722.353	77.261	*.000
temp * frq	765.259	54, 378	14.171	. 981	. 516

Note. temp = temperature; frq = frequency; * $p < 0.01$.

Table 3.

Tests of Within-Subjects Effects results of ANOVA for DPOAEs on TG2.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
int_fin	1674.082	1, 7	1674.082	9.426	.018
freq	22085.799	18, 126	1226.989	36.419	*.000
int_fin * freq	152.924	18, 126	8.496	.718	.787

Note. int = initial; fin = final; freq = frequency; * $p < 0.01$.

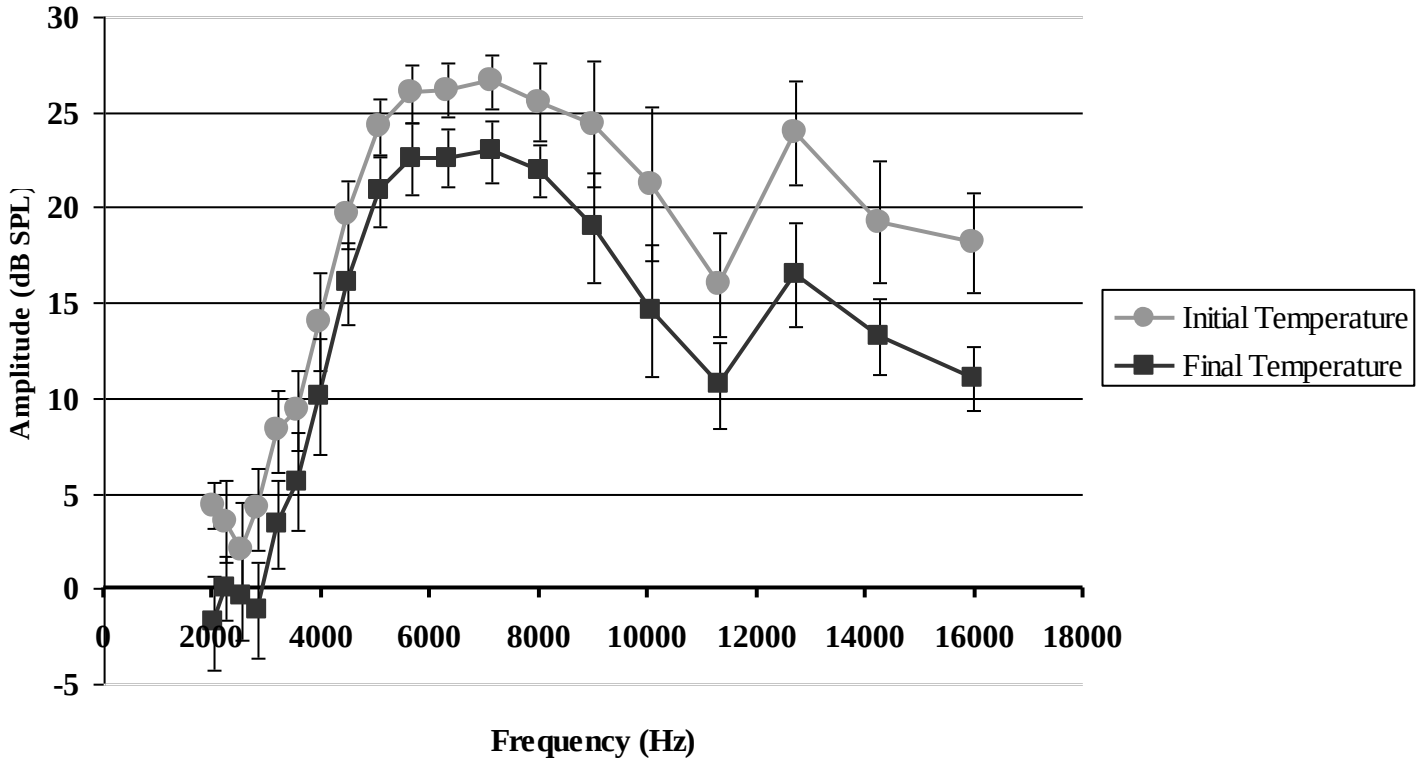

Figure 3. DPOAE results (averages for 8 rats ± SEM) for initial and final measurements performed at a temperature of 37.5ºC for trial group 2.

Table 4.

Tests of Within-Subjects Effects and Between-Subjects Effects results of ANOVA for DPOAEs on initial measurements (body temperature of 37.5ºC in both cases) for TG1 and TG2.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
TG	250.288	1, 14	250.288	314.950	.375
freq	23538.861	18	1307.714	66.732	*.000
freq * TG	149.138	18, 252	8.285	.423	.982

Note. temp = temperature; frq = frequency; * $p < 0.01$.

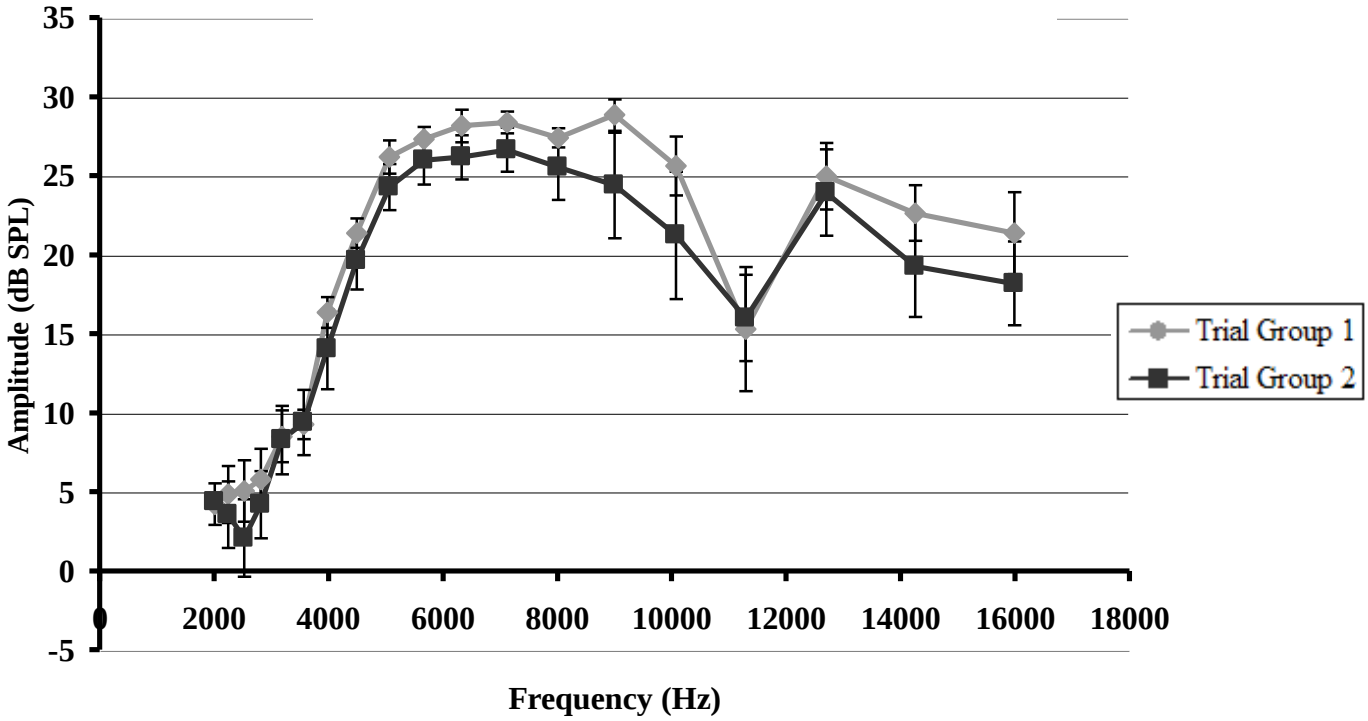

Figure 4. DPOAE results (averages for 8 rats in each group ± SEM) for initial measurements performed at the same temperature of 37.5ºC for trial groups 1 and 2.

Table 5.

Comparison of DPOAEs for initial measurements (body temperature of 37.5ºC in both cases) for TG1 and TG2 across all test frequencies.

F2 (Hz)	Levene's Test for Equality of Variances F	Sig.	t	df	*p* (2-tailed)
15984	.111	.745	.859	14	.405
14250	4.126	.062	.925	14	.371
12703	.823	.380	.305	14	.765
11296	.595	.453	-.146	14	.886
*10078	8.912	*.010	.988	14	.340
9000	6.236	.026	1.276	14	.223
*8015	15.240	*.002	.882	14	.393
7125	2.469	.138	1.117	14	.283
6328	.503	.490	1.156	14	.267
5671	1.599	.227	.778	14	.449
5062	.055	.817	1.069	14	.303
4500	.616	.446	.843	14	.414
3984	1.409	.255	.860	14	.404
3562	1.441	.250	-.063	14	.951
3187	.390	.542	.086	14	.933
2812	.039	.846	.560	14	.584
2531	.006	.938	.947	14	.359
2250	.044	.837	.463	14	.650
2015	.370	.552	-.086	14	.932

Note. * $p < 0.01$.

homogeneity of the data was determined at all frequencies except at 8,015 Hz and 10,078 Hz in the mid-frequency range (Table 5).

The differences in DPOAE amplitude measured at baseline body temperature (37.5ºC) and at each of the three higher temperatures (38.5ºC, 39.5ºC, and 40.5ºC) were calculated. The mean differences for each trial group (TG1, TG2) for 38.5ºC, 39.5ºC, and 40.5ºC compared to baseline are presented in Figures 5, 6, and 7, respectively, with ± standard error measurements (SEM) for amplitude differences at each temperature. Examination of these figures demonstrates that the amplitude differences between temperatures did not exceed 6 dB at any frequency for any temperature for either trial group. The largest temperature differences occurred for TG1 above 10,000 Hz at 38.5ºC and 39.5ºC and below 10,000 Hz at 40.5ºC. At these locations the amplitude differences of TG1 and TG2 did not overlap, but for the remaining ranges, the amplitude differences for TG1 and TG2 were essentially the same. The amplitude differences were similar between TG1 and TG2 except at these locations where amplitude differences did not overlap. The differences seen across trial group were not statistically significant, as shown by a test of between-subjects effects (Table 6). The data were collapsed across all sixteen rats for all further analyses.

After the data from the sixteen rats were collapsed, the differences in amplitude between the baseline temperature and the three temperatures 38.5ºC, 39.5ºC, and 40.5ºC was calculated and the results are shown in Figures 8, 9, and 10, respectively. Mean amplitude differences did not exceed 3 dB at any frequency. The largest differences (approximately 3 dB) were seen at 2,015 Hz and 11,296 Hz for 38.5ºC, and from 7,000 to 9000 Hz and at 12,703 Hz for 40.5ºC. A 4x19 (temperature x frequency) repeated

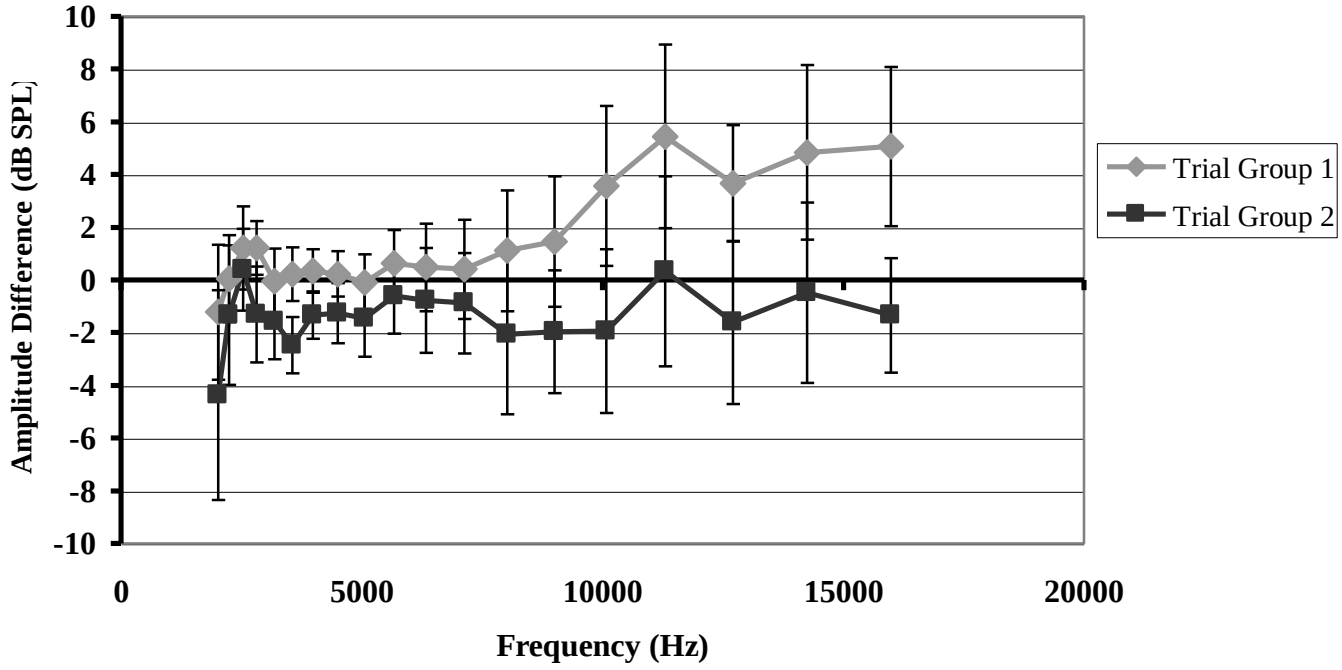

Figure 5. DPOAE amplitude difference results (averages for 8 rats in each group ± SEM) between baseline temperature and measurements performed at a temperature of 38.5ºC for trial groups 1 and 2.

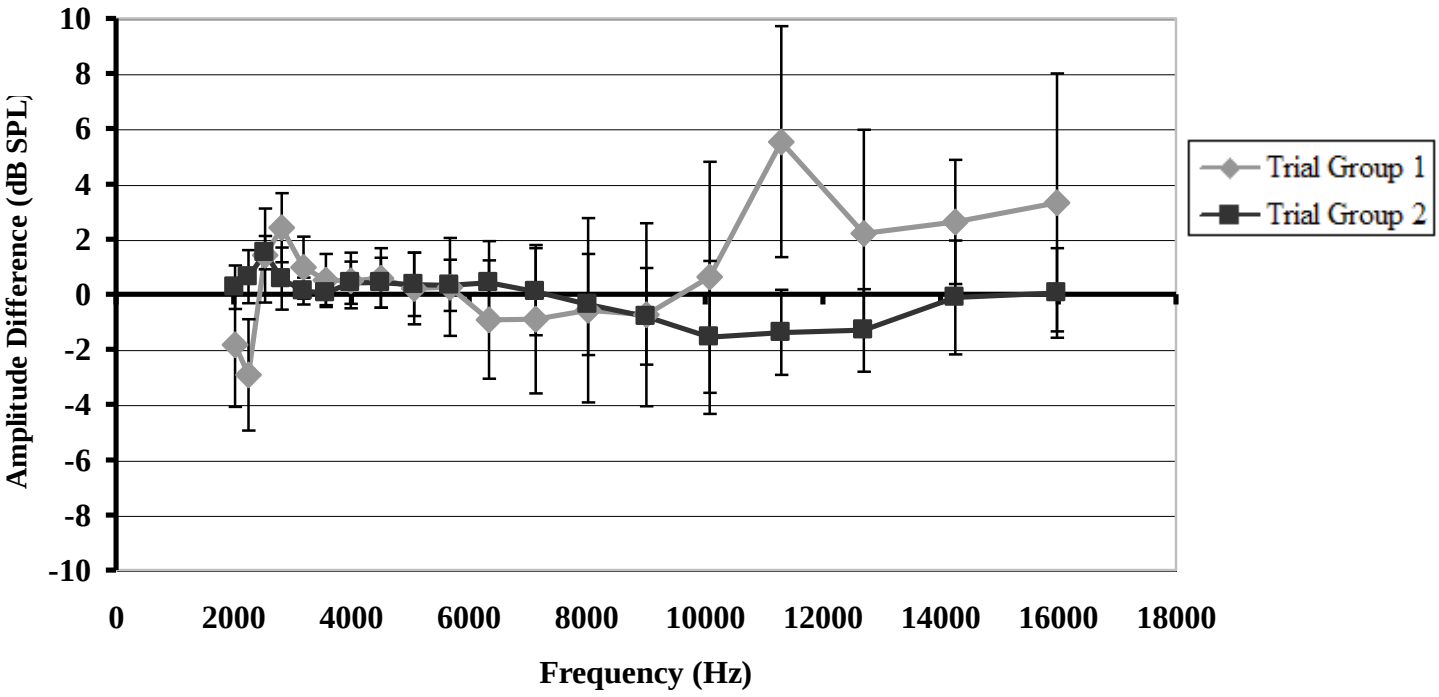

Figure 6. DPOAE amplitude difference results (averages for 8 rats in each group ± SEM) between baseline temperature and measurements performed at a temperature of 39.5ºC for trial groups 1 and 2.

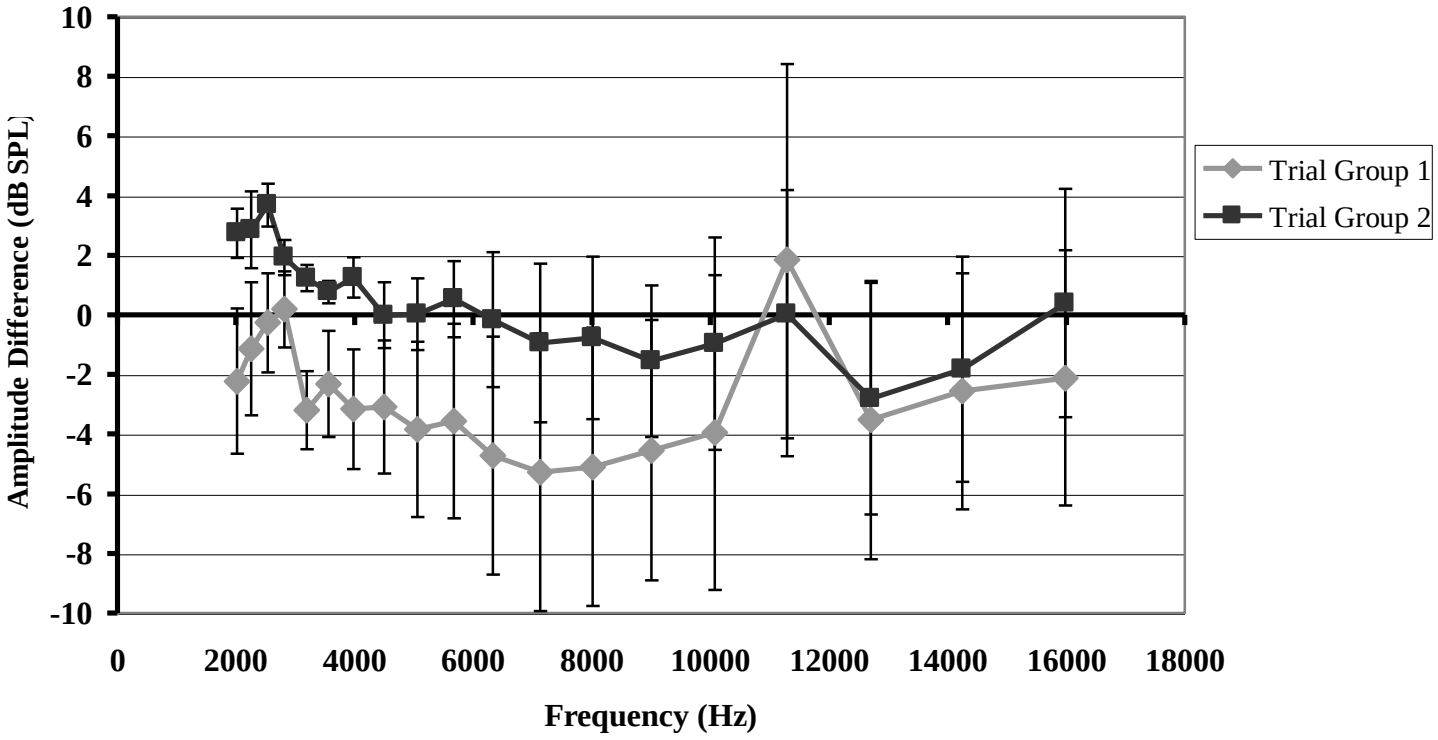

Figure 7. DPOAE amplitude difference results (averages for 8 rats in each group ± SEM) between baseline temperature and measurements performed at a temperature of 40.5ºC for trial groups 1 and 2.

Table 6.

Tests of Between-Subjects Effects results of ANOVA for DPOAEs.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
TG	1140.418	1, 14	1140.418	.760	.398

Note. TG = Trial Group; * $p < 0.01$.

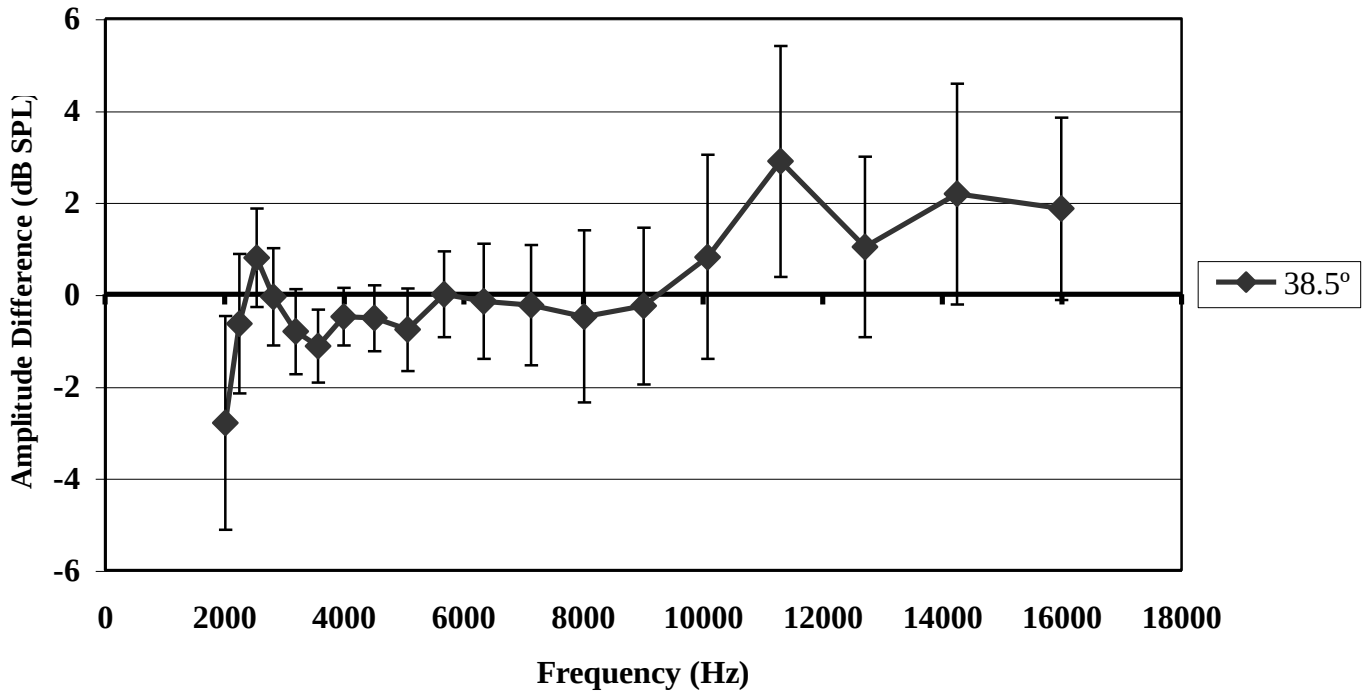

Figure 8. DPOAE amplitude difference results (averages for 16 rats ± SEM) between baseline temperature and measurements performed at a temperature of 38.5ºC.

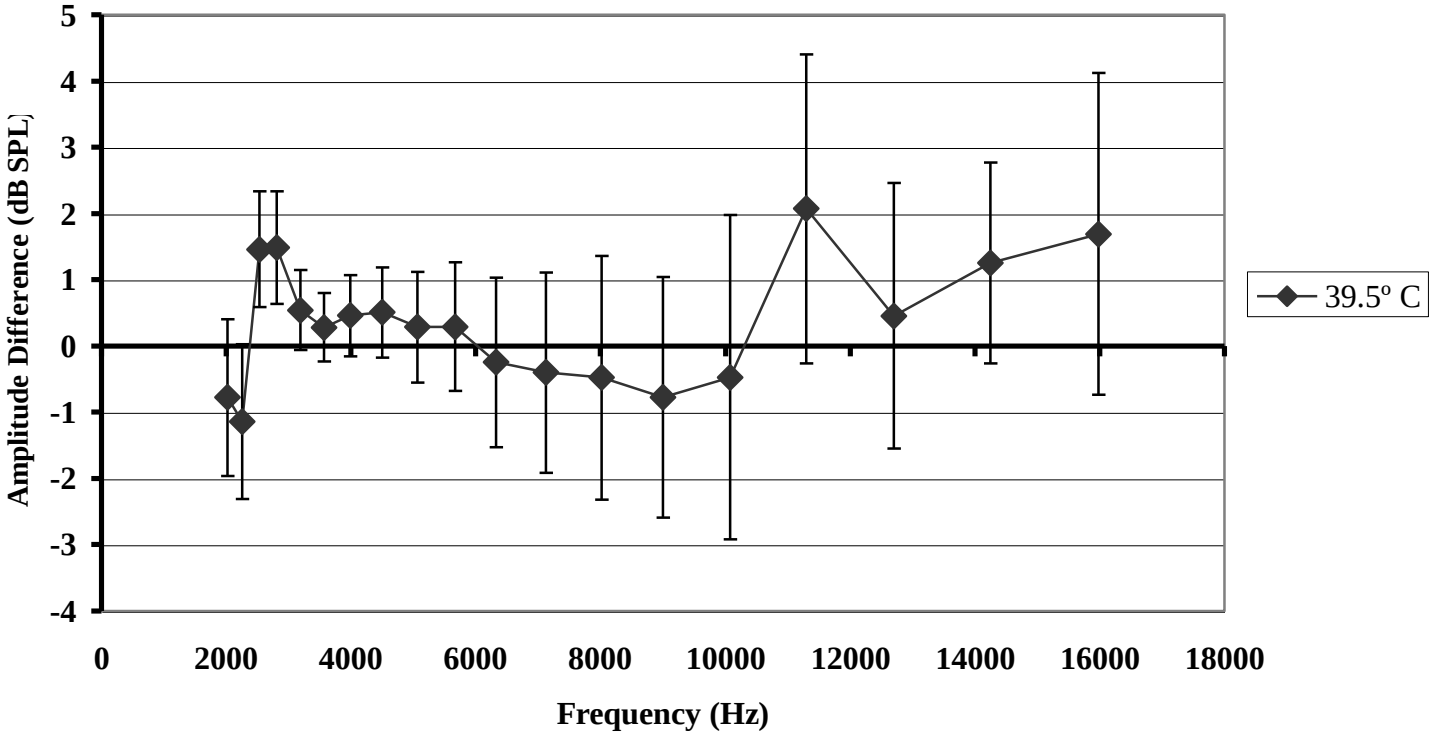

Figure 9. DPOAE amplitude difference results (averages for 16 rats ± SEM) between baseline temperature and measurements performed at a temperature of 39.5ºC.

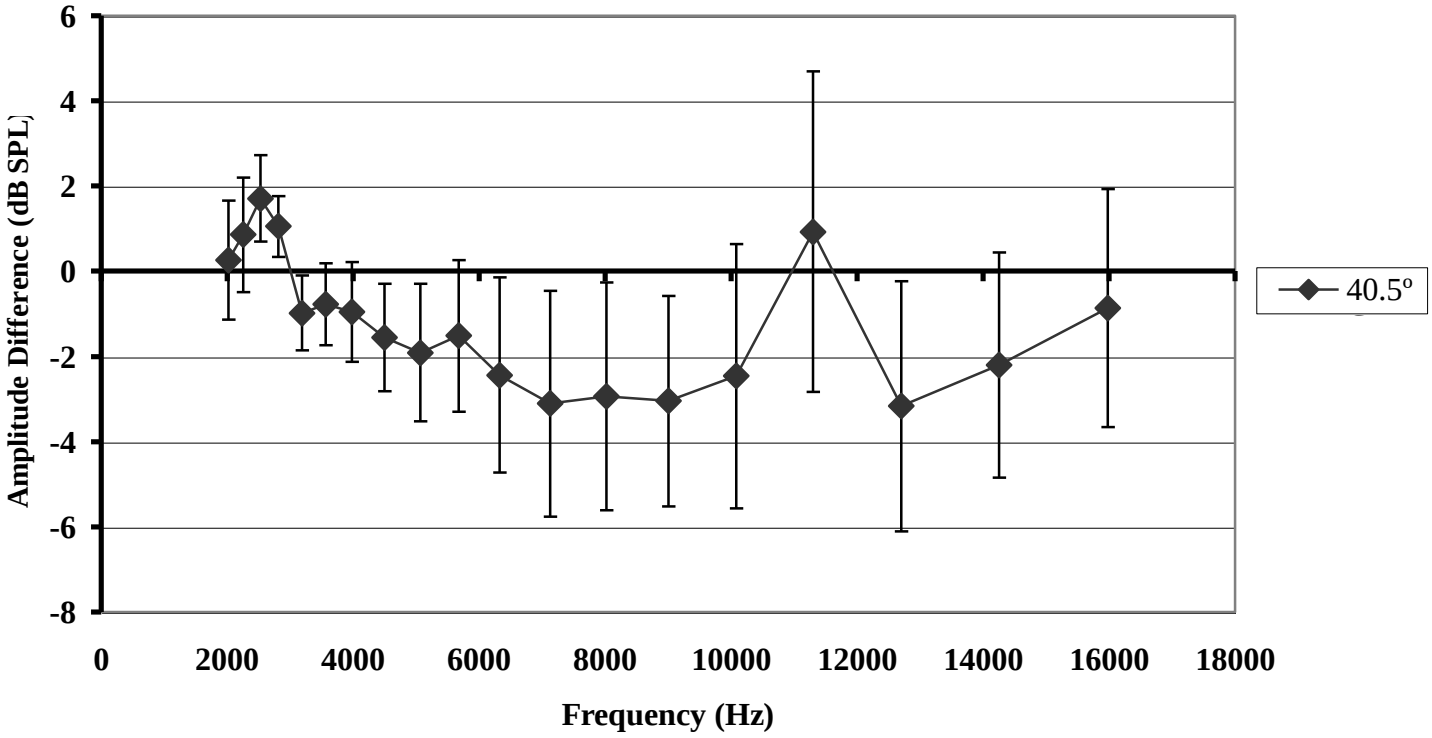

Figure 10. DPOAE amplitude difference results (averages for 16 rats ± SEM) between baseline temperature and measurements performed at a temperature of 40.5ºC.

measures analysis of variance was conducted to compare temperature and frequency across all sixteen rats. There was a significant main effect for frequency, F (18, 270) = 86.872, $p = 0.000$, but not for temperature or the interaction between temperature and frequency (Table 7). Mean amplitude differences for all three temperatures are displayed on Figure 11 for direct comparison. Examination of this figure indicates the mean amplitude difference was greatest in magnitude (on the order of -3 dB, indicating a decrease in amplitude) at the highest temperature (40.5ºC) compared with the two lower temperatures. For the two lower temperatures, 38.5ºC and 39.5ºC, the amplitude difference was positive (indicating an increase in amplitude), but only by 1-2 dB and only for frequencies above 10,000 Hz.

Auditory Brainstem Responses

The mean ABR thresholds across frequency for each temperature are illustrated in Figure 12 for TG1 and Figure 13 for TG2. For both groups, examination of these figures for all four temperatures indicated that the average ABR threshold was approximately 60 dB at 3,150 Hz, then slightly increased to between 70 and 80 dB at the next three frequencies and returned to approximately 60 dB at 20,000 Hz. This pattern was similar for TG1 and TG2.

A 4x5 (temperature x frequency) repeated measures analysis of variance was conducted for TG1. There was a significant main effect for frequency F (4, 28) = 25.370, $p = 0.000$, but not for temperature or the interaction between temperature and frequency (Table 8). A 5x5 (temperature x frequency) repeated measures analysis of variance was also conducted on TG2. There was also a significant main effect for temperature F (4,

Table 7.

Tests of Within-Subjects Effects results of ANOVA for DPOAEs on all temperatures for TG1 and TG2.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
temp	436.535	3, 45	145.512	.459	.712
frq	88383.453	18, 270	4910.192	86.872	*.000
temp * frq	866.524	54, 810	16.047	1.374	.042

Note. temp = temperature; frq = frequency; * $p < 0.01$.

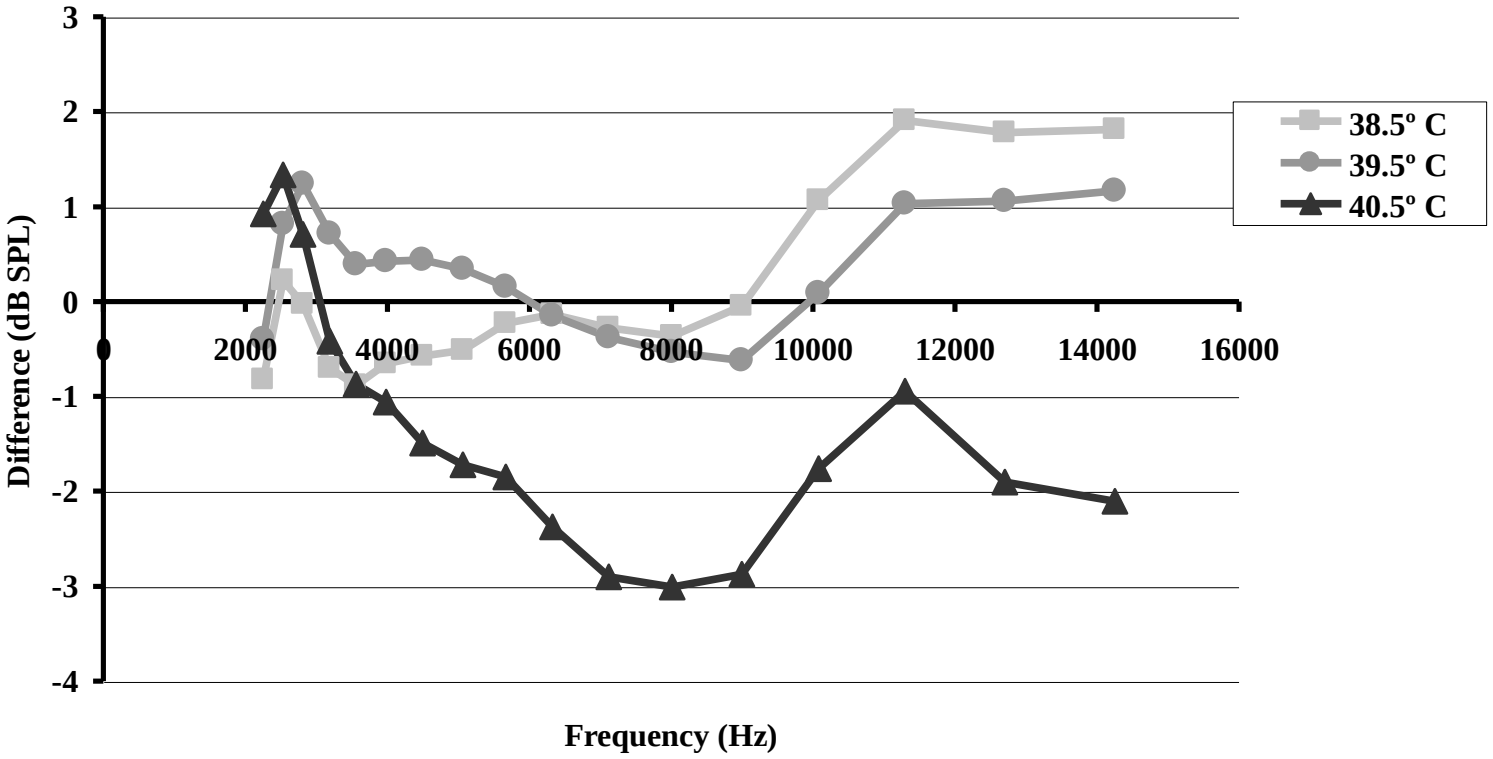

Figure 11. Smoothed DPOAE differences (averages for 16 rats ± SEM) for measurements performed at temperatures of 38.5ºC, 39.5ºC, and 40.5ºC.

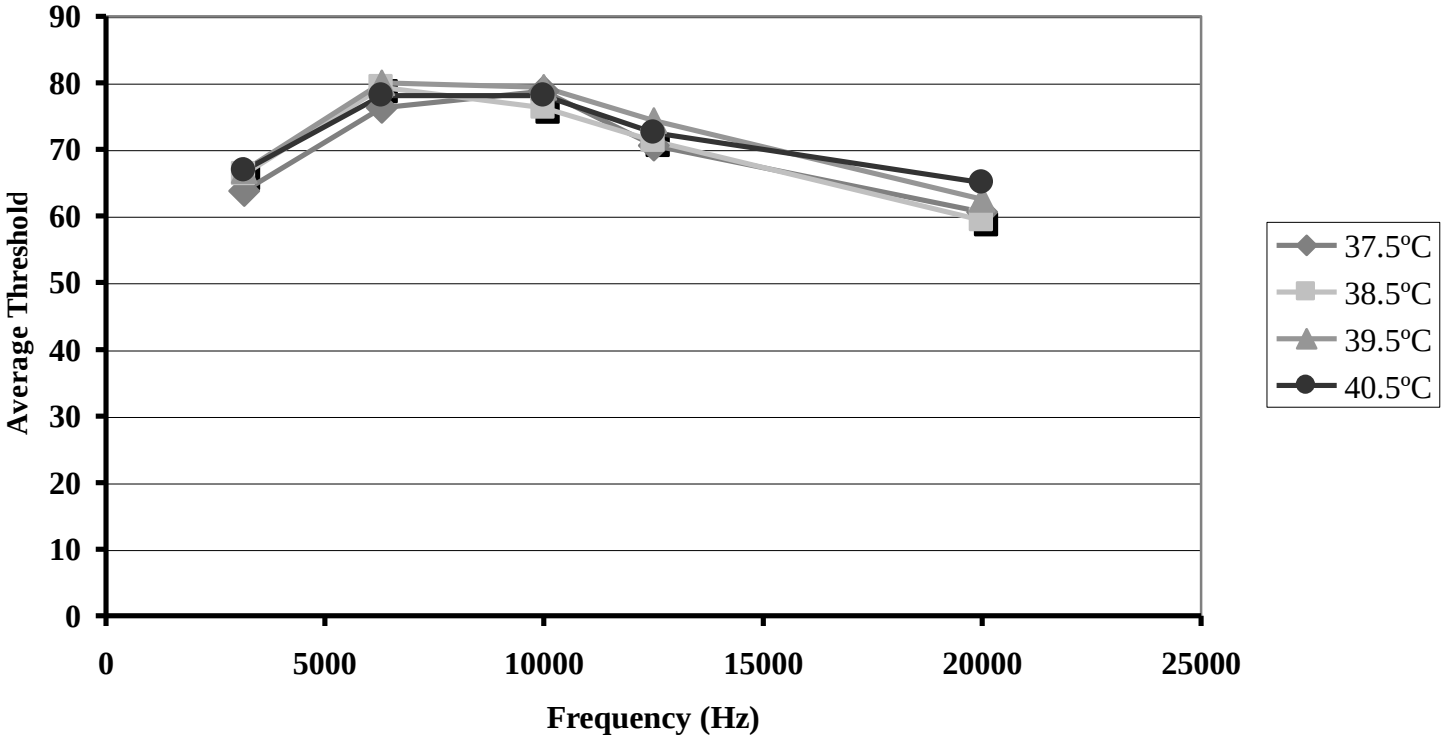

Figure 12. ABR results (averages for 8 rats) for measurements performed at temperatures of 37.5ºC, 38.5ºC, 39.5ºC and 40.5ºC for trial group 1.

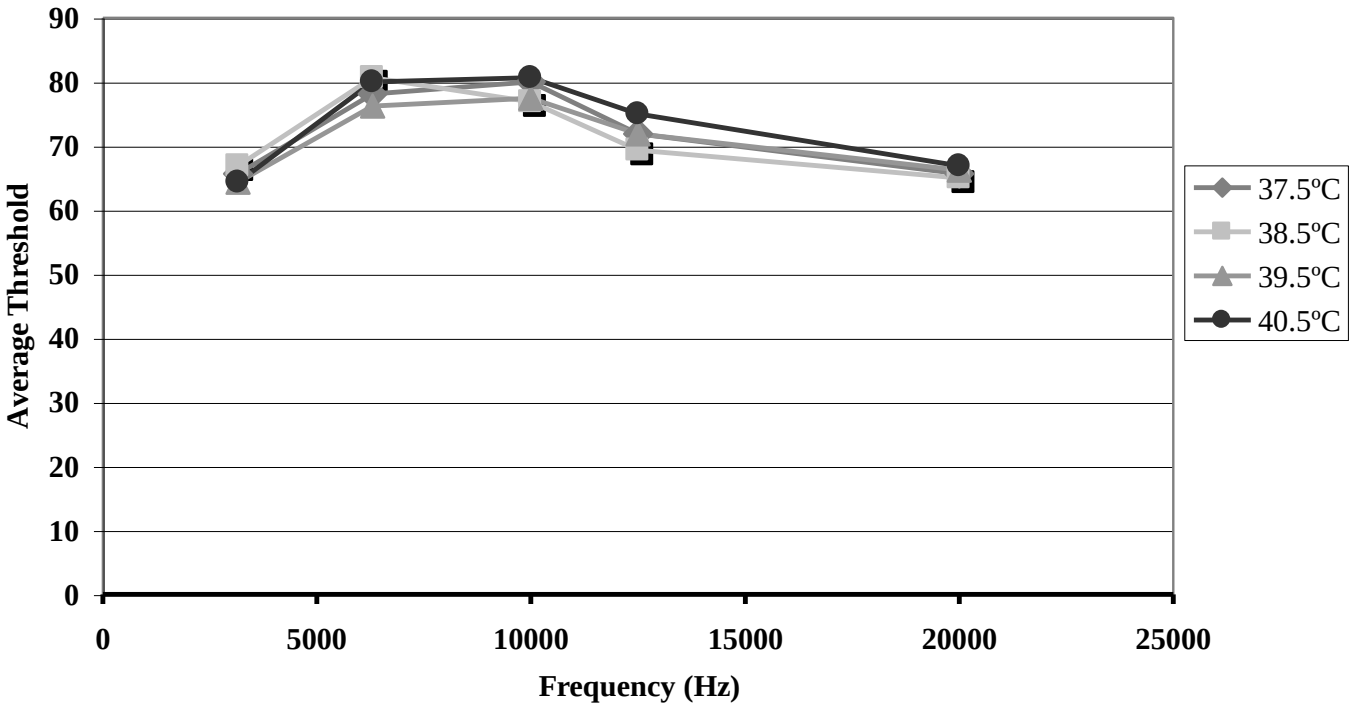

Figure 13. ABR results (averages for 8 rats) for measurements performed at temperatures of 37.5ºC, 38.5ºC, 39.5ºC and 40.5ºC for trial group 2.

Table 8.

Tests of Within-Subjects Effects results of ANOVA for ABRs on TG1.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
temp	190.625	3, 21	63.542	1.139	.356
freq	6908.750	4, 28	1727.188	25.370	*.000
temp * freq	181.250	12, 84	15.104	.462	.931

Note. temp = temperature; frq = frequency; * $p < 0.01$.

28) = 10.458, $p = 0.000$, but not for temperature or the interaction between temperature and frequency (Table 9).

A 2x5 (trial x frequency) repeated measures analysis of variance was conducted to determine the reliability of the 37.5ºC ABR measurements for TG2, when the measurements were taken at the beginning of data collection (initial baseline temperature) and after all other temperatures had been tested (final temperature/returned to baseline temperature). The results of this ANOVA indicated a significant main effect for frequency F (4, 28) = 9.371, $p = 0.000$, but not for trial or the interaction between trial and frequency (Table 10). The results are presented in Figure 14.

A 2x5 (group x frequency) mixed model analysis of variance was conducted to compare the ABR threshold at an initial body temperature of 37.5ºC for TG1 to the ABR threshold at the same initial body temperature of 37.5ºC for TG2. The results of this analysis indicated a significant main effect for frequency F (4) = 19.905, $p = 0.000$, but no significant effect for trial group or the interaction between trial group and frequency (Table 11) indicating the groups appeared to be equal at baseline for ABR threshold and temperature. The results are presented in Figure 15.

Independent sample *t* tests were conducted at all frequencies to compare the ABR threshold obtained for trial group 1 versus trial group 2 at the initial body temperature of 37.5ºC. As shown in Table 11, the results of the independent *t* tests revealed there was no significant difference in ABR thresholds for group 1 versus group 2 at any of the test frequencies. Based on a Levene's test of equality of variance, homogeneity of the data was determined at all frequencies (see Table 12).

Table 9.

Tests of Within-Subjects Effects results of ANOVA for ABRs on TG2.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
temp	369.500	4, 28	92.375	1.977	.126
freq	6300.750	4, 28	1575.188	10.458	*.000
temp * freq	393.000	16, 112	24.563	1.171	.302

Note. temp = temperature; frq = frequency; * $p < 0.01$.

Table 10.

Tests of Within-Subjects Effects results of ANOVA for initial and final temperatures for ABRs on TG2.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Int_fin	180.000	1, 7	180.000	5.143	.058
freq	2258.125	4, 28	564.531	9.371	*.000
Int_fin * freq	123.125	4, 28	30.781	.905	.474

Note. int – initial; fin – final; frq = frequency; * $p < 0.01$.

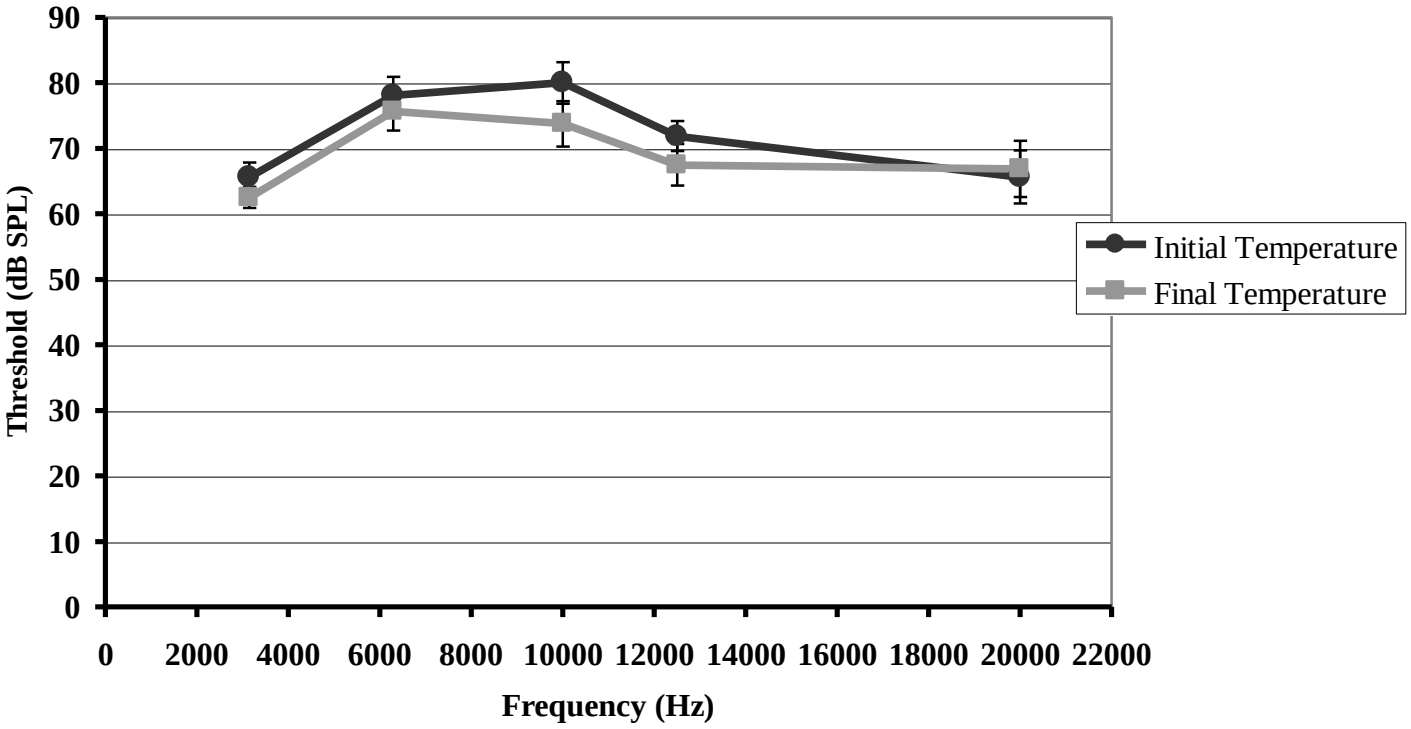

Figure 14. ABR results (averages for 8 rats ± SEM) for initial and final measurements performed at the same temperature of 37.5ºC for trial group 2.

Table 11.

Tests of Within-Subjects Effects and Between-Subjects Effects results of ANOVA for ABRs on initial measurements (body temperature of 37.5ºC in both cases) for TG1 and TG2.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
TG	101.250	1, 14	101.250	.493	.494
freq	3364.375	4	841.094	19.905	*.000
freq * TG	39.375	4, 56	9.844	.233	.919

Note. temp = temperature; frq = frequency; * $p < 0.01$.

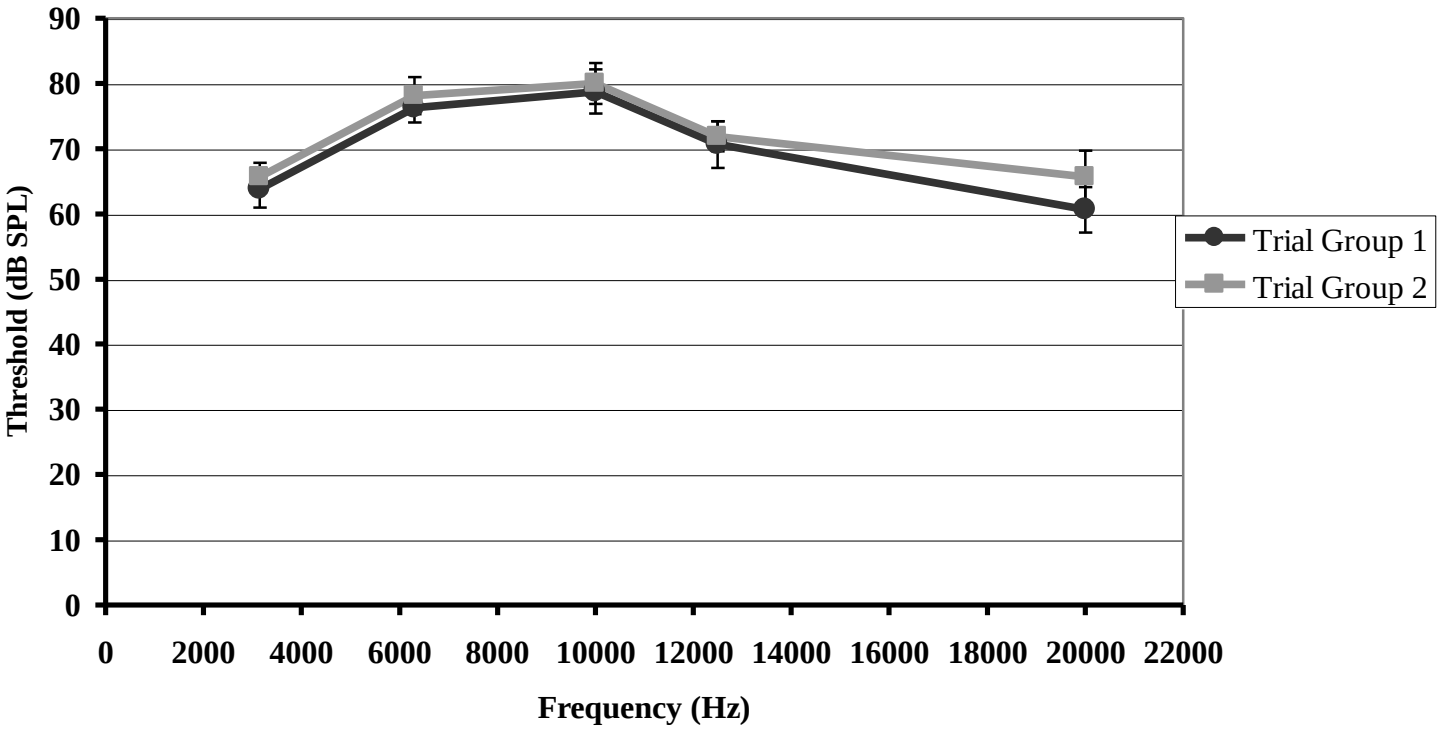

Figure 15. ABR results (averages for 8 rats in each group ± SEM) for initial measurements performed at the same temperature of 37.5ºC for trial groups 1 and 2.

Table 12.

Comparison of ABRs for initial measurements (body temperature of 37.5ºC in both cases) for TG1 and TG2 across all test frequencies.

F2 (Hz)	Levene's Test for Equality of Variances F	Sig.	t	df	*p* (2-tailed)
3,150	.175	.682	-.527	14	.607
6,300	.282	.604	-.518	14	.613
10,000	.022	.884	-.271	14	.790
12,500	1.474	.245	-.293	14	.774
20,000	.196	.665	-.937	14	.365

The differences in ABR thresholds that were measured at the current temperature versus the initial baseline temperature were calculated for all three temperatures (38.5ºC, 39.5ºC, and 40.5ºC) by subtracting the ABR thresholds at each temperature from the ABR thresholds at 37.5ºC (baseline body temperature). This difference calculation was made for the recordings at 38.5ºC, 39.5ºC, and 40.5ºC. The results for TG1 and TG2 at 38.5ºC, 39.5ºC, and 40.5ºC are presented in Figures 16, 17, and 18, respectively, with ± standard error measurements (SEM) for threshold differences at each temperature. The mean threshold differences for each temperature did not exceed about 4 dB at any frequency for any temperature for either trial group. The mean amplitude differences were similar across trial group. The largest difference between trial groups (approximately 6 dB) was seen at 6,300 Hz for 39.5ºC. A test of between-subjects effects showed no significant difference between the two trial groups (Table 13). The data were collapsed across all sixteen rats for further analysis.

After the data from the sixteen rats were collapsed, the mean differences in thresholds at all three temperatures 38.5ºC, 39.5ºC, and 40.5ºC was calculated and the results are shown in Figures 19, 20, and 21, respectively. Examination of these figures indicates the mean threshold difference was no greater than 3 dB. The greatest differences (on the order of 2 dB) were seen at 6,300 Hz and 10,000 Hz for 38.5ºC, 12,500 at 39.5ºC and above 20,000 Hz for 40.5ºC. A 4x5 (temperature x frequency) repeated measures analysis of variance was conducted to compare temperature and frequency across all sixteen rats. There was a significant main effect for frequency, $F(4, 60) = 30.024$, $p = 0.000$, but not for temperature or the interaction between temperature and frequency (Table 14). Threshold differences for all three temperatures are smoothed

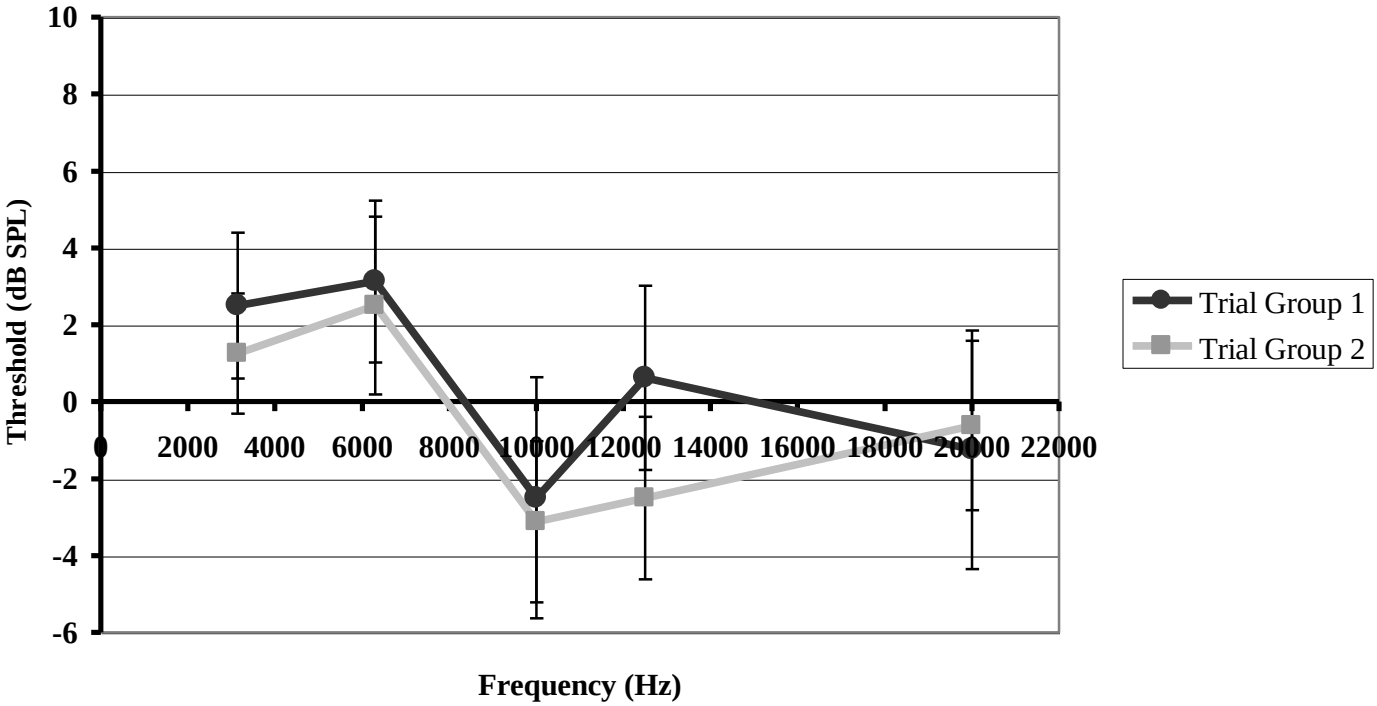

Figure 16. ABR threshold difference results (averages for 8 rats in each group ± SEM) between baseline temperature and measurements performed at a temperature of 38.5ºC for trial groups 1 and 2.

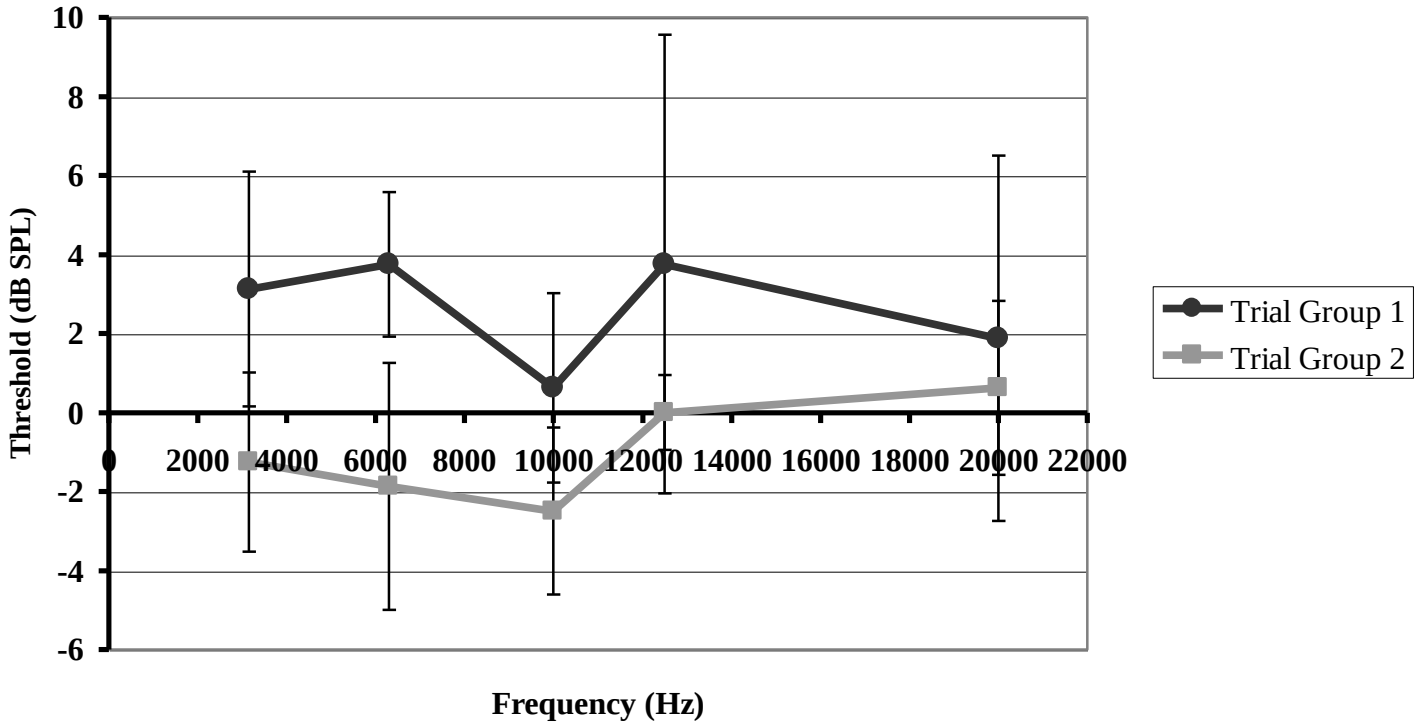

Figure 17. ABR threshold difference results (averages for 8 rats in each group ± SEM) between baseline temperature and measurements performed at a temperature of 39.5ºC for trial groups 1 and 2.

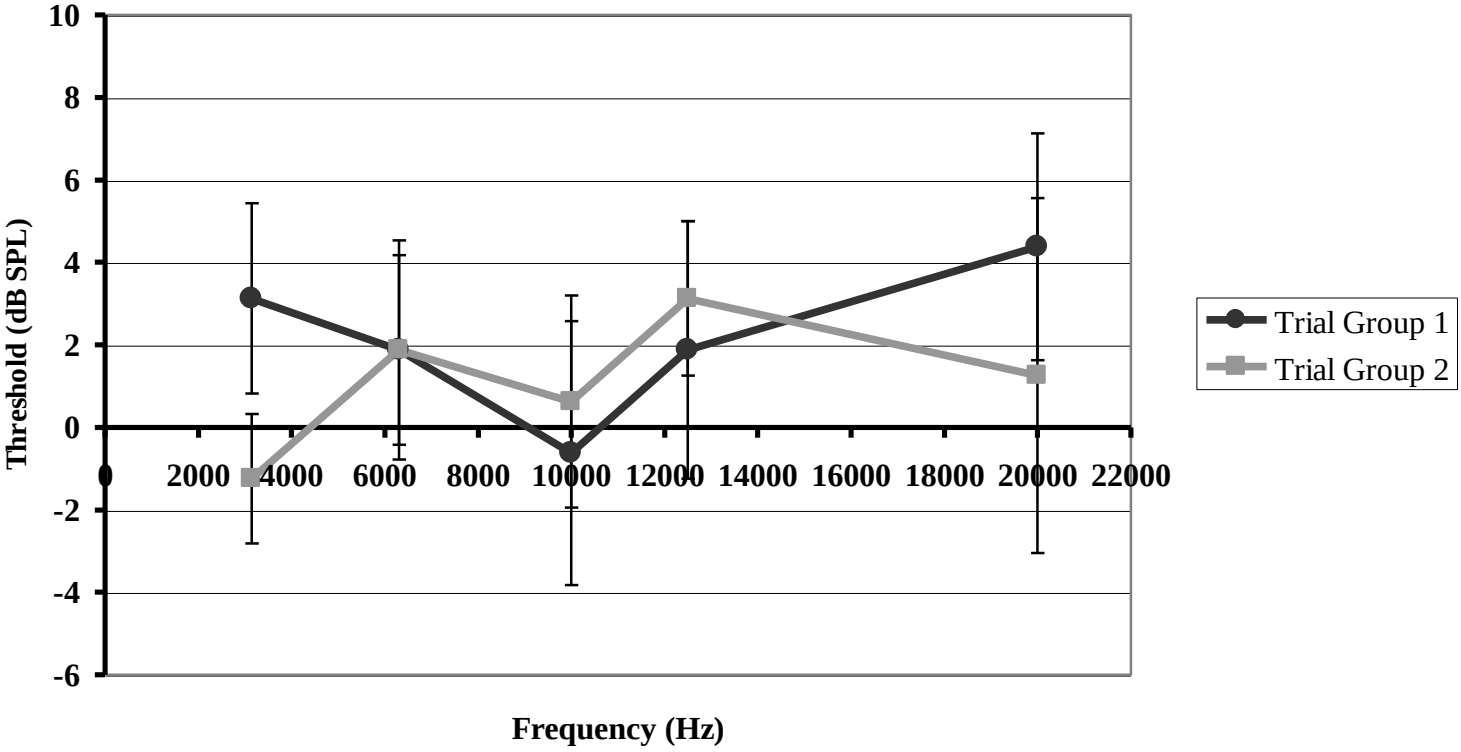

Figure 18. ABR threshold difference results (averages for 8 rats in each group ± SEM) between baseline temperature and measurements performed at a temperature of 40.5ºC for trial groups 1 and 2.

Table 13.

Tests of Between-Subjects Effects results of ANOVA for ABRs.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
TG	56.953	1, 14	56.953	.141	.713

Note. TG = Trial Group; * $p < 0.01$.

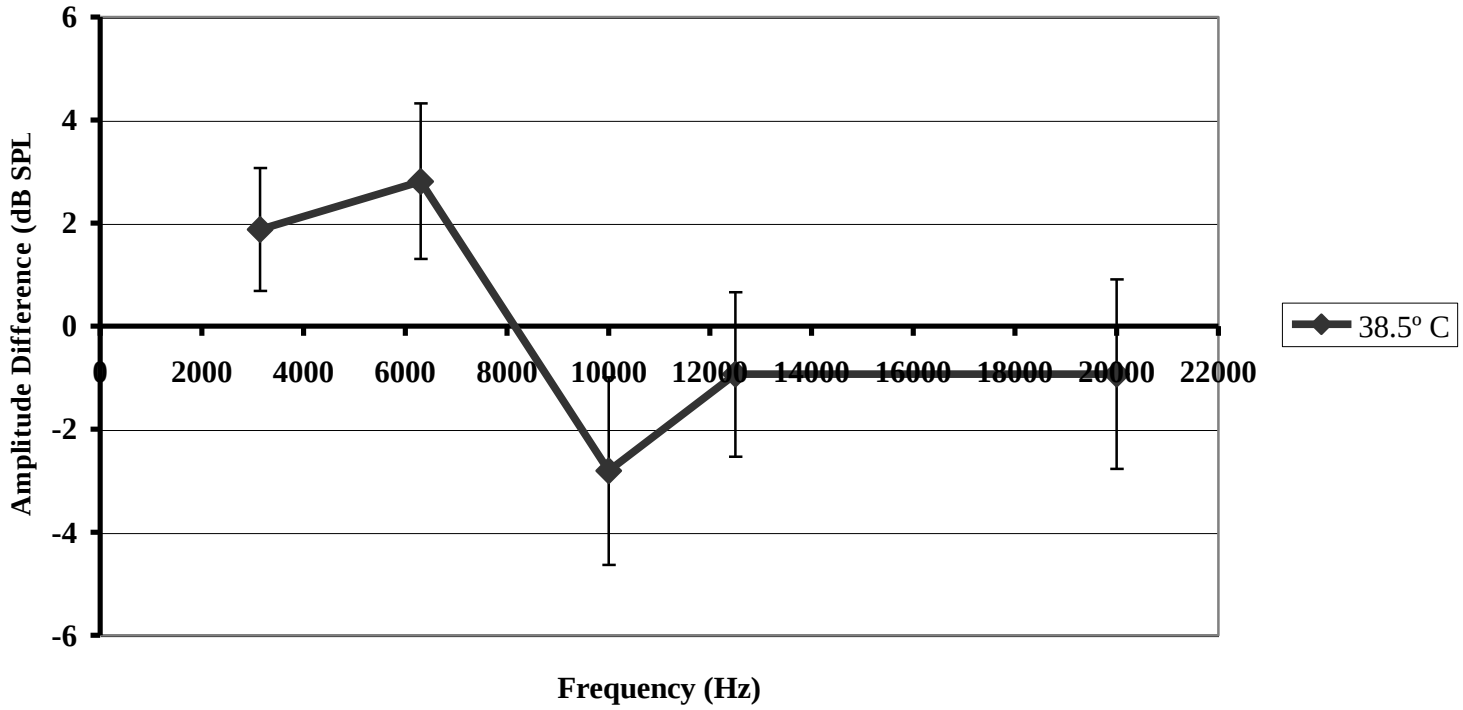

Figure 19. ABR threshold difference results (averages for 16 rats ± SEM) between baseline temperature and measurements performed at a temperature of 38.5ºC.

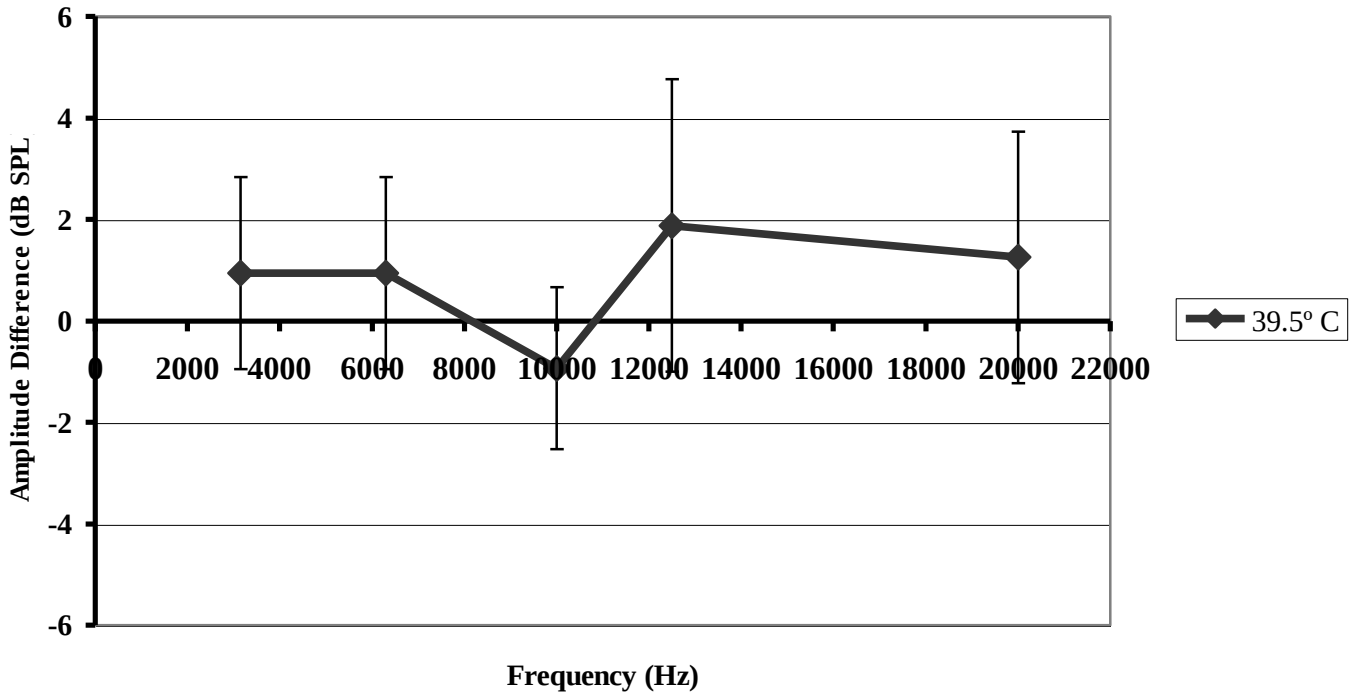

Figure 20. ABR threshold difference results (averages for 16 rats ± SEM) between baseline temperature and measurements performed at a temperature of 39.5ºC.

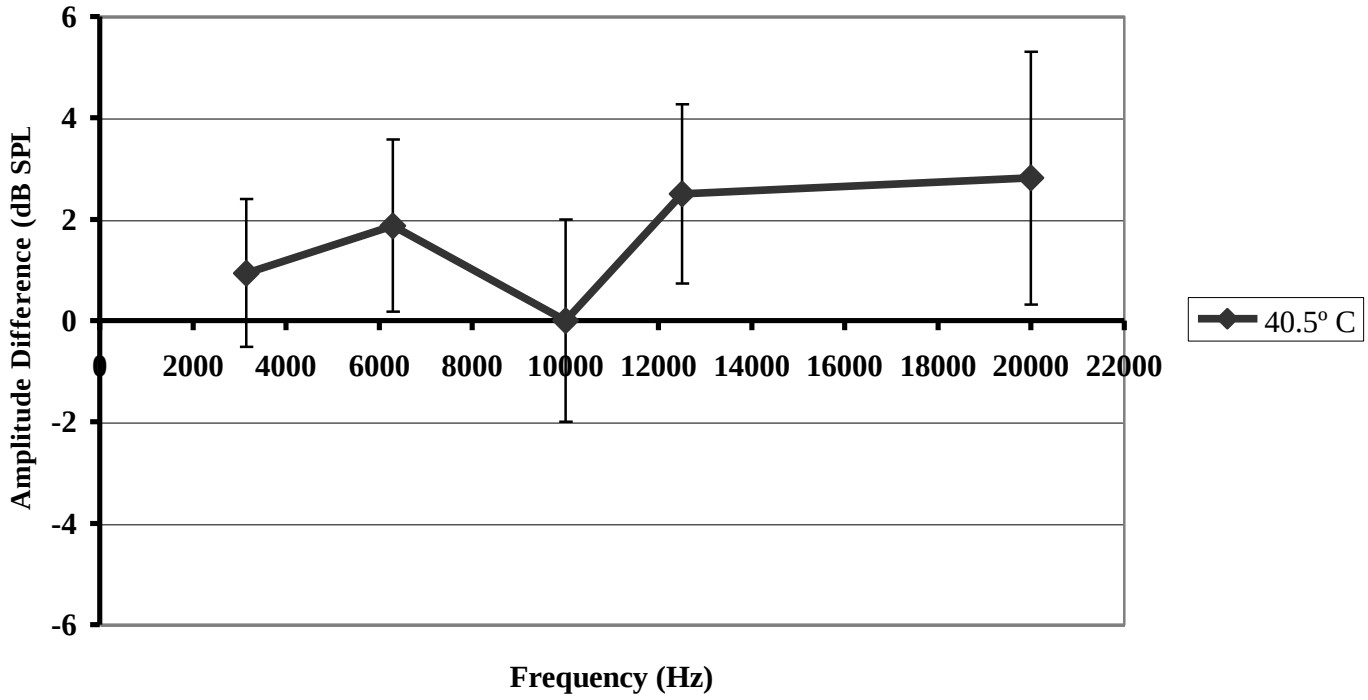

Figure 21. ABR threshold difference results (averages for 16 rats ± SEM) between baseline temperature and measurements performed at a temperature of 40.5ºC.

Table 14.

Tests of Within-Subjects Effects results of ANOVA for ABRs on all temperatures for TG1 and TG2.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
temp	145.234	3, 45	48.411	.942	.428
freq	12206.563	4, 60	3051.641	30.024	*.000
temp * freq	287.188	12, 180	23.932	1.002	.449

Note. temp = temperature; freq = frequency; * $p < 0.01$.

and displayed on Figure 22 for comparison. Figure 22 makes visualization of the data easier to compare by smoothing the fluctuations and preserving the original structure of the data. The smoothed graph was calculated by using 25% of the threshold value of the previous data point, 50% of current data point, and 25% of the following data point to generate a smoothed data point.

DPOAE and ABR Regression Analysis

A regression analysis was conducted comparing the threshold differences across temperatures (38.5ºC, 39.5ºC, and 40.5ºC) at five ABR frequencies with the DPOAE amplitude differences across temperature at the most closely matched DPOAE F2 frequency (Table 15). The differences in DPOAE amplitude at each frequency (3.15k, 6.3k, 10k, 12.5k, and 20k Hz) for all sixteen rats across all three temperatures (38.5ºC, 39.5ºC, and 40.5ºC) were plotted against the differences in ABR threshold for each frequency (Figures 23-27). The regression analysis (R^2 value and model) for each frequency is included in each graph. The regression analysis indicated a low and non-significant R^2 value at each frequency (Table 16), indicating that the amount of variance predicted by the best-fit model was not significant. The differences in DPOAE amplitude at each temperature (38.5ºC, 39.5ºC, and 40.5ºC) for all sixteen rats across all five frequencies (3.15k, 6.3k, 10k, 12.5k, and 20k Hz) were plotted against the differences in ABR threshold at each temperature (Figures 28-30). The regression analysis (R^2 value and model) for each temperature is included with each graph. The regression analysis indicated a low and non-significant R^2 value at each frequency (Table 17), indicating that the amount of variance predicted by the best-fit model was not significant.

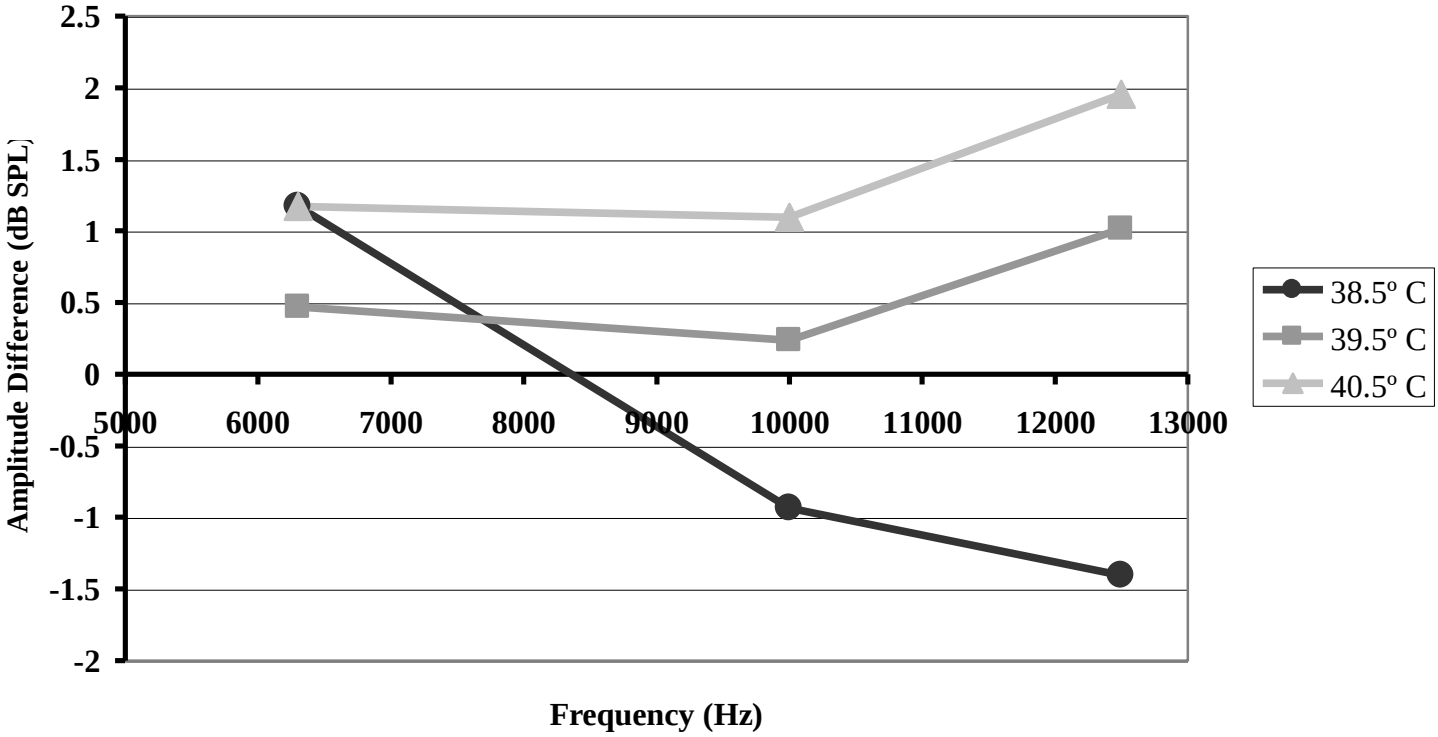

Figure 22. Smoothed ABR threshold differences (averages for 16 rats ± SEM) for measurements performed at temperatures of 38.5ºC, 39.5ºC, and 40.5ºC.

Table 15.

ABR frequencies paired with the F2 DPOAE frequency for regression analysis.

ABR Frequency (Hz)	DPOAE Frequency (Hz)
3,150	3,187.50
6,300	6,328.125
10,000	10,078.13
12,500	12,703.13
20,000	15,984.38

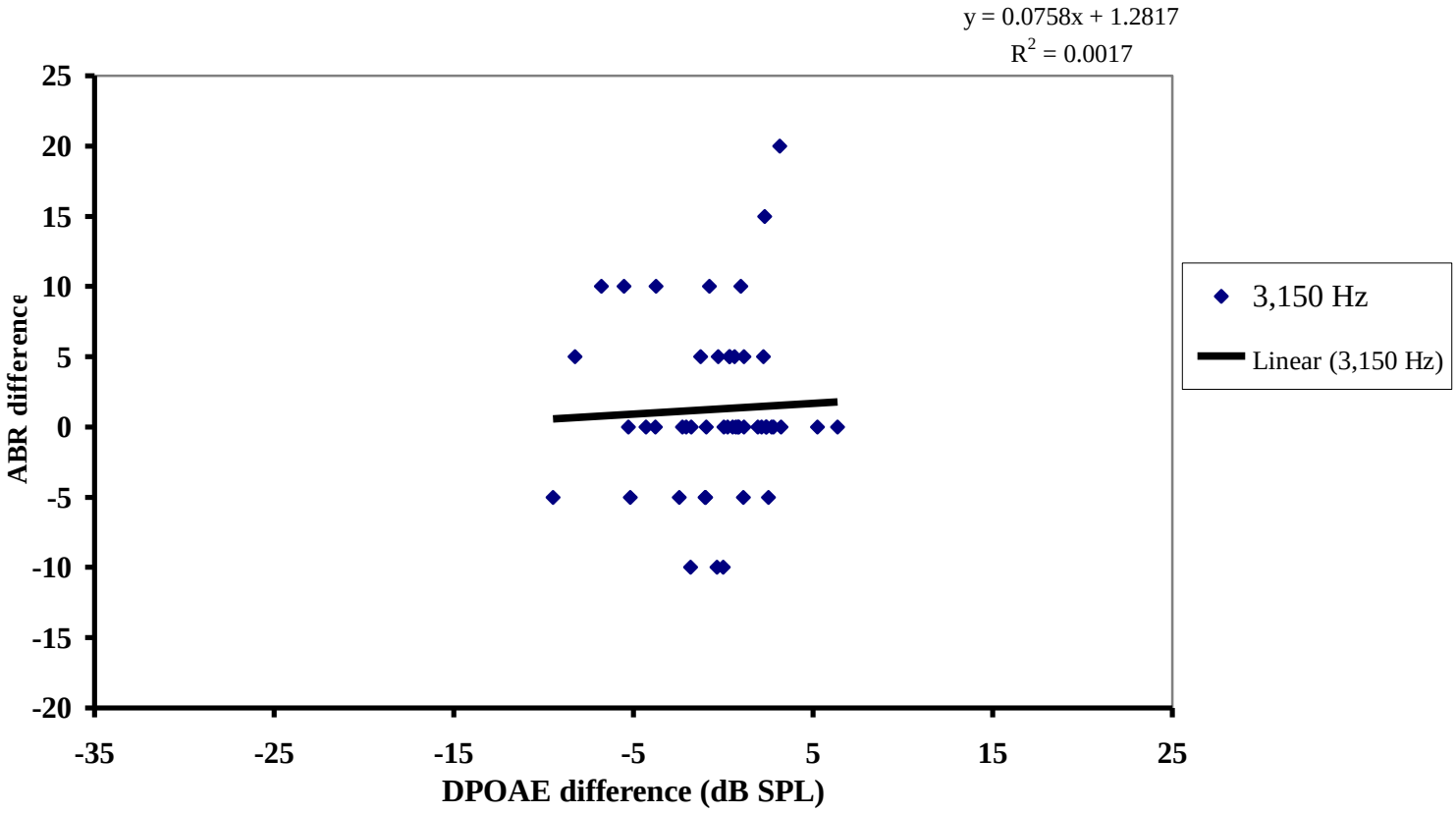

Figure 23. Regression analysis of DPOAEs and ABRs (averages for 16 rats) for measurements performed at 3,150 Hz for temperatures of 38.5ºC, 39.5ºC, and 40.5ºC.

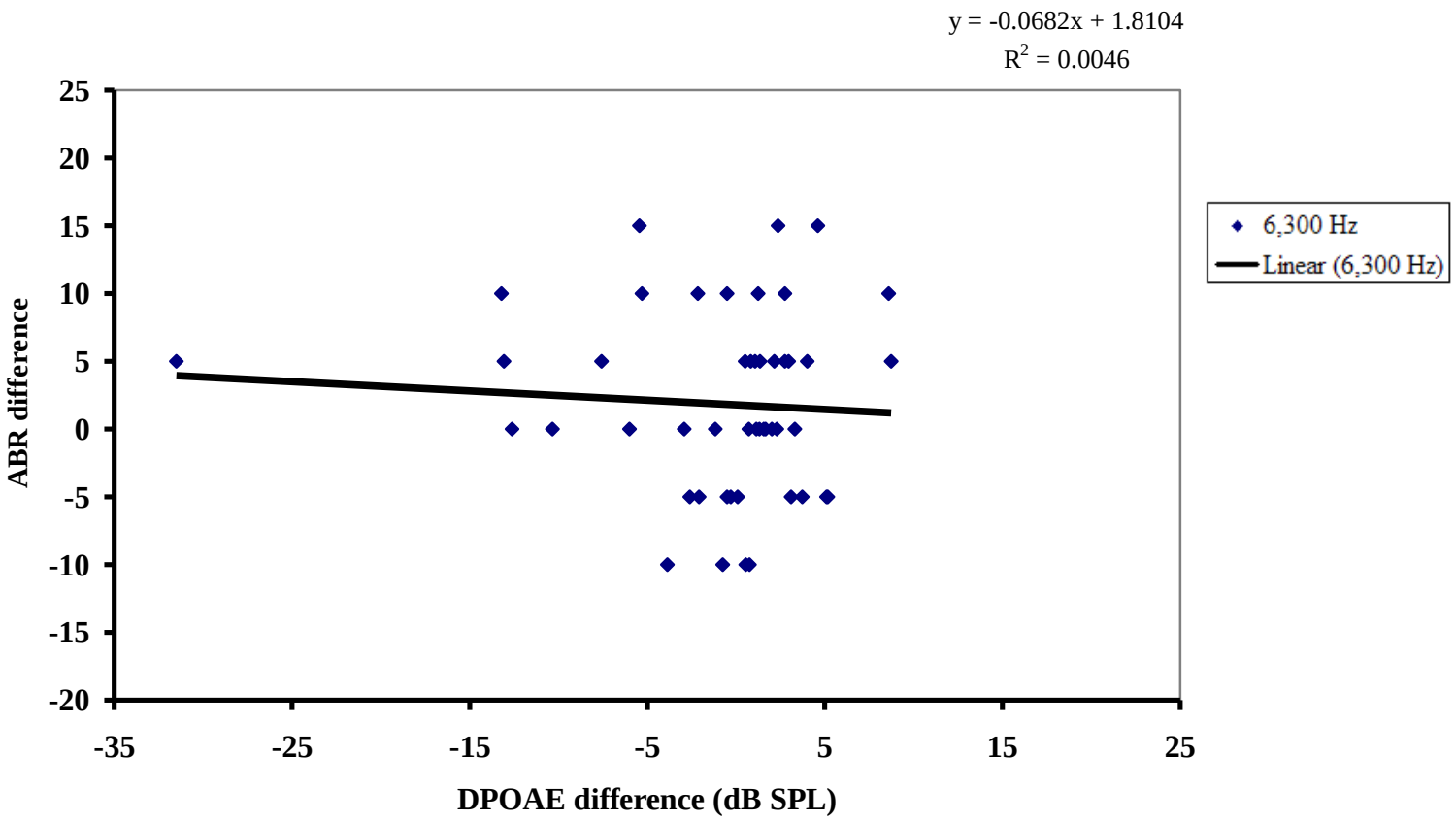

Figure 24. Regression analysis of DPOAEs and ABRs (averages for 16 rats) for measurements performed at 6,300 Hz for temperatures of 38.5ºC, 39.5ºC, and 40.5ºC.

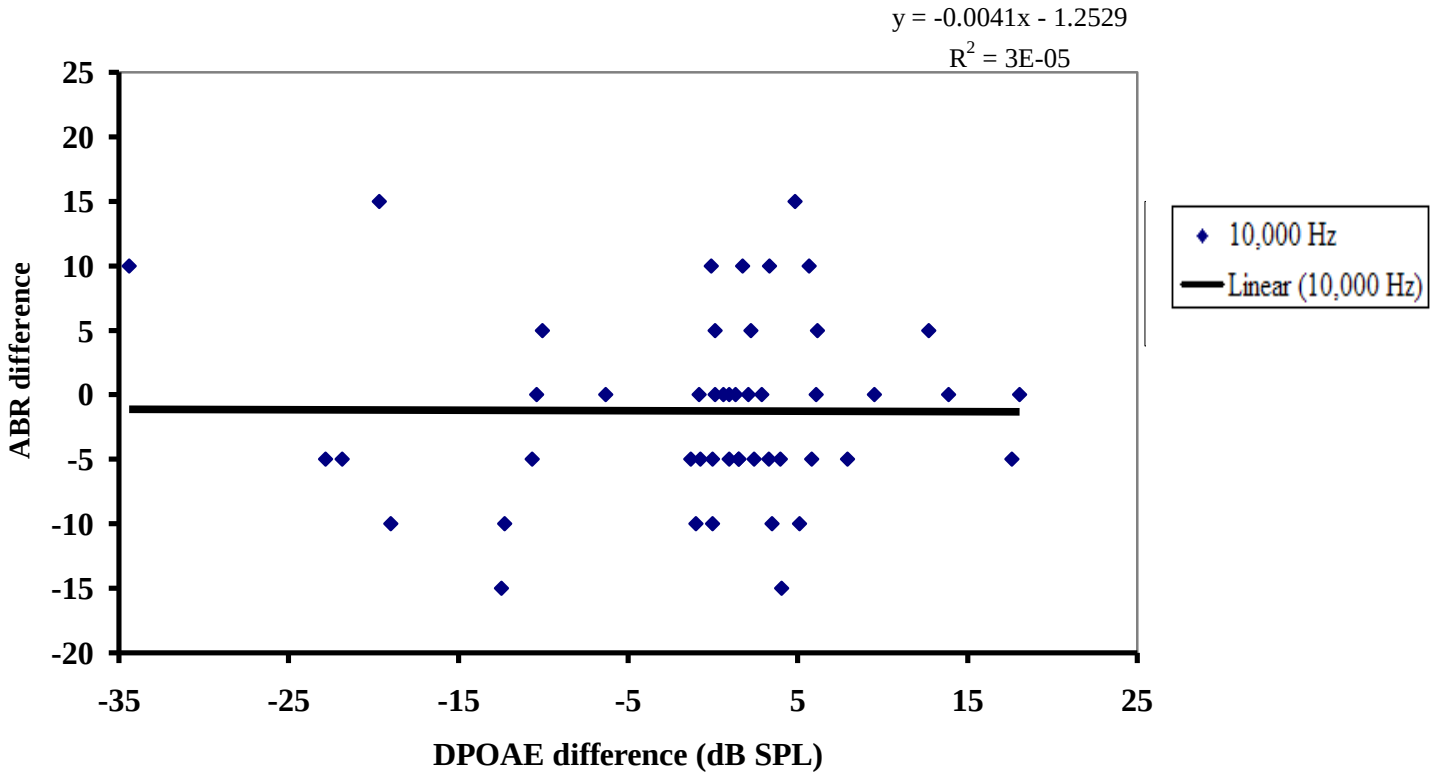

Figure 25. Regression analysis of DPOAEs and ABRs (averages for 16 rats) for measurements performed at 10,000 Hz for temperatures of 38.5ºC, 39.5ºC, and 40.5ºC.

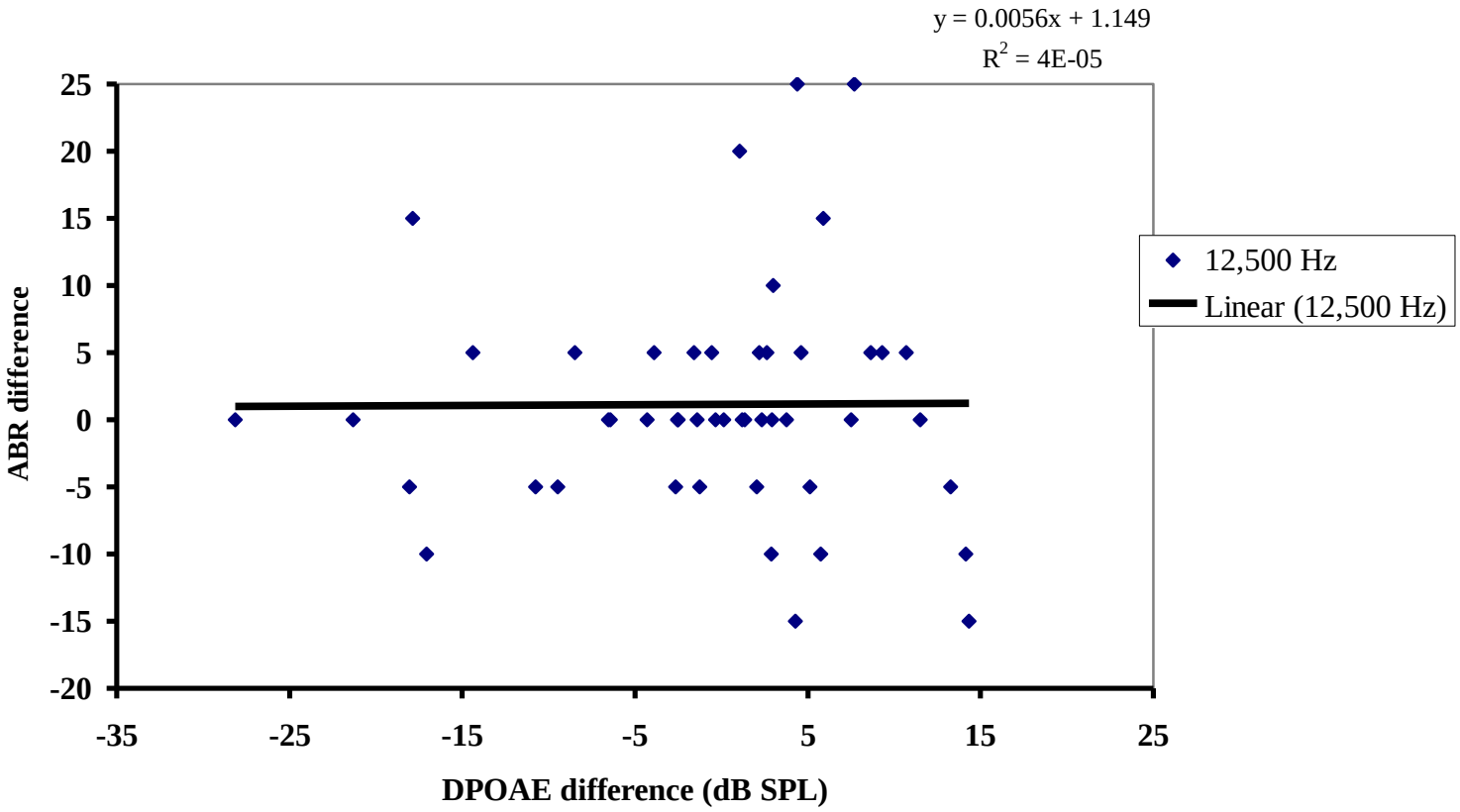

Figure 26. Regression analysis of DPOAEs and ABRs (averages for 16 rats) for measurements performed at 12,500 Hz for temperatures of 38.5ºC, 39.5ºC, and 40.5ºC.

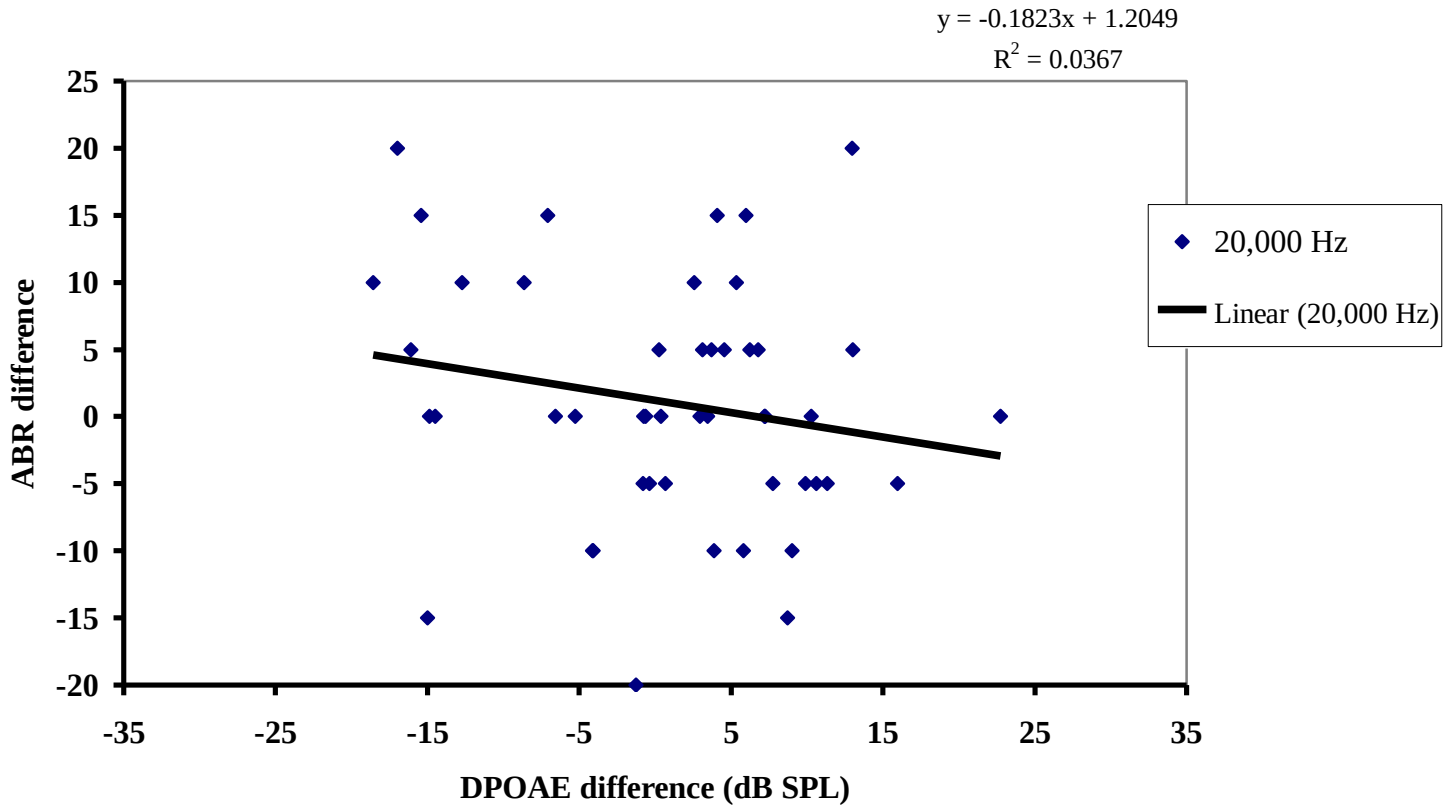

Figure 27. Regression analysis of DPOAEs and ABRs (averages for 16 rats) for measurements performed at 20,000 Hz for temperatures of 38.5ºC, 39.5ºC, and 40.5ºC.

Table 16.

Regression analysis by frequency of ABR frequencies paired with F2 DPOAE frequencies.

Frequency (Hz)	r^2
3,150	0.0017
6,300	0.0046
10,000	0.00003
12,500	0.00004
20,000	0.0367

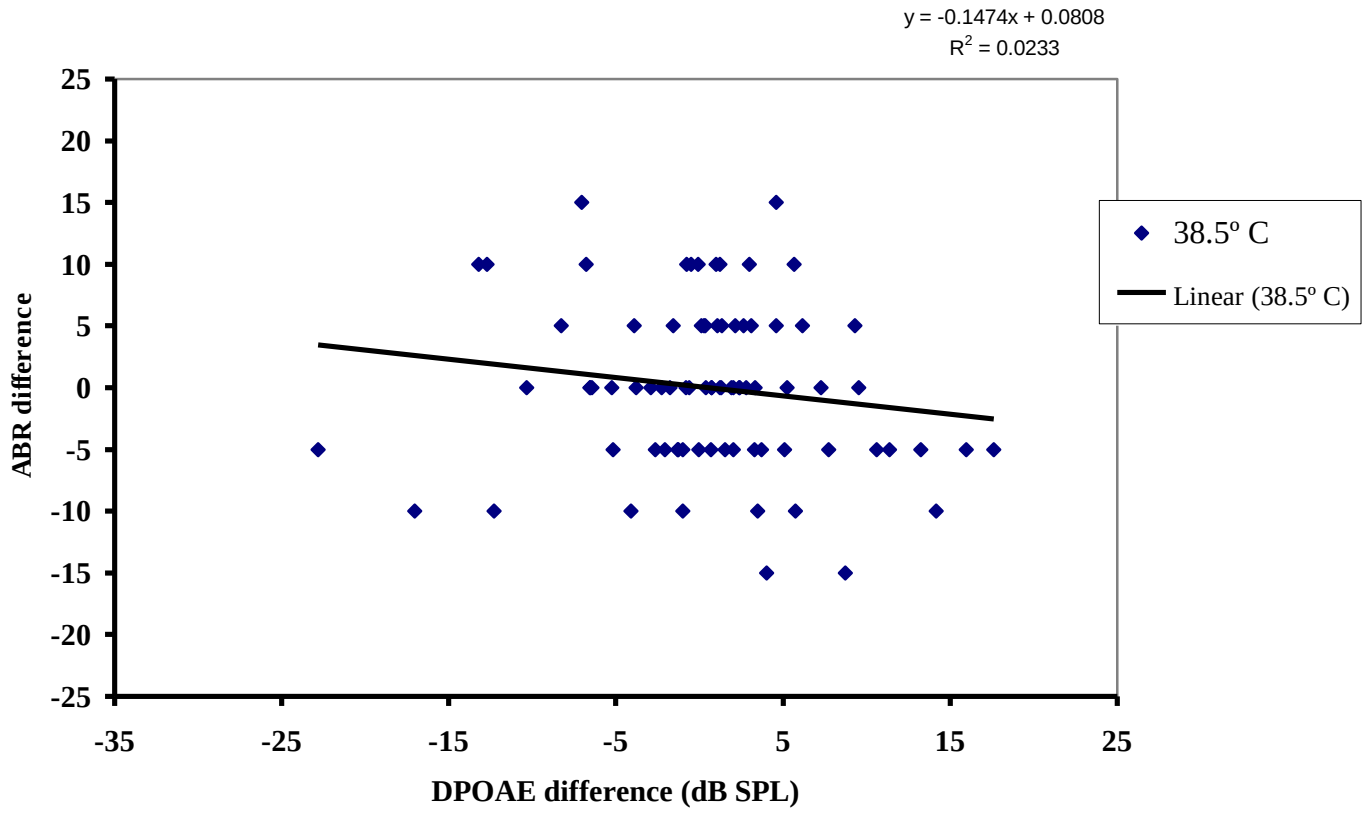

Figure 28. Regression analysis of DPOAEs and ABRs (averages for 16 rats) for measurements performed at 38.5ºC for the five frequencies listed in Table 19.

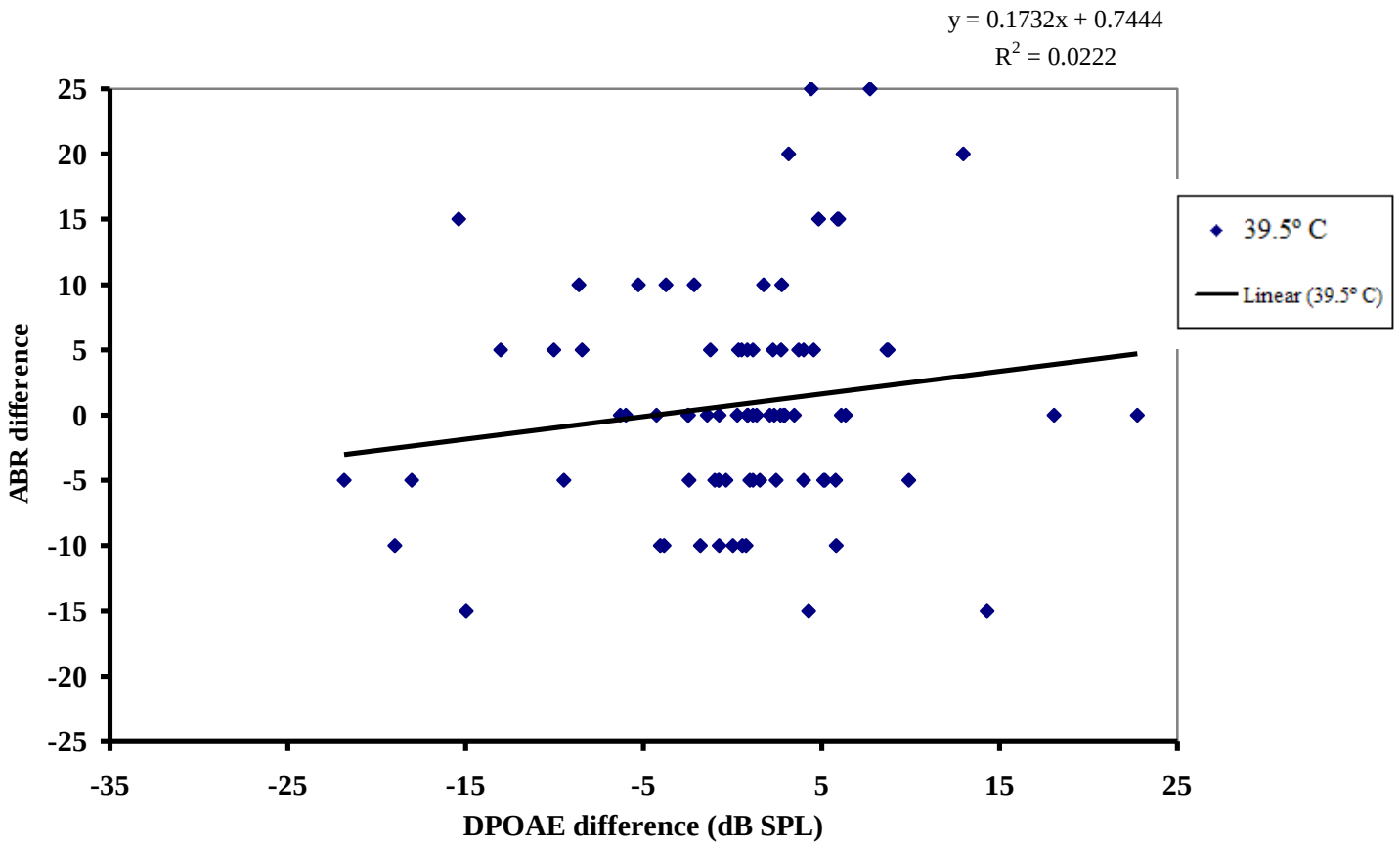

Figure 29. Regression analysis of DPOAEs and ABRs (averages for 16 rats) for measurements performed at 39.5ºC for the five frequencies listed in Table 19.

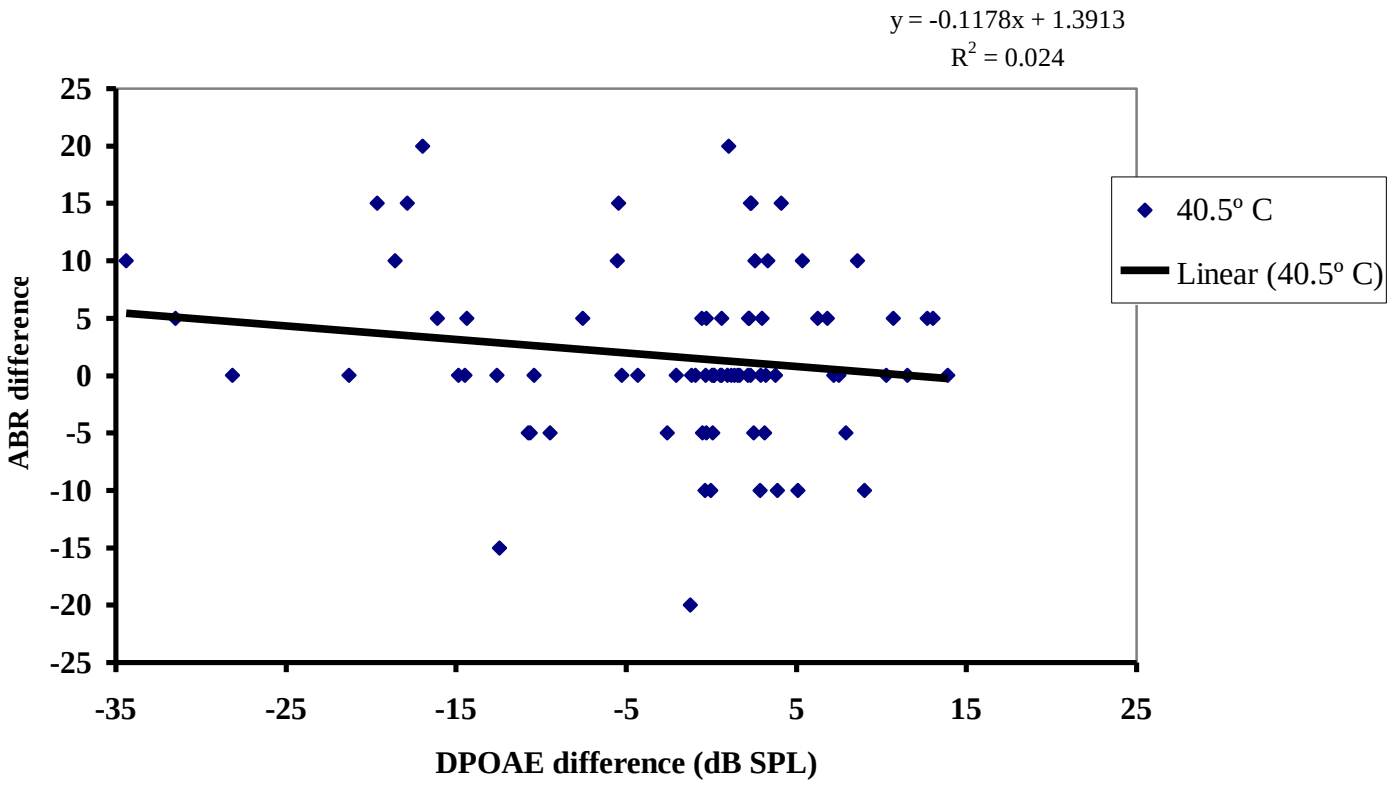

Figure 30. Regression analysis of DPOAEs and ABRs (averages for 16 rats) for measurements performed at 40.5ºC for the five frequencies listed in Table 19.

Table 17.

Regression analysis by temperature of ABR frequencies paired with F2 DPOAE frequencies.

Temperature (ºC)	r^2
38.5ºC	0.0233
39.5ºC	0.0222
40.5ºC	0.024

CHAPTER 5

DISCUSSION

It has been shown in both animals and humans that even small changes in body temperature may cause changes in otoacoustic emission amplitude as well as changes in the amplitude and latency of the auditory brainstem response. Hypothermic changes down 33.41°C and hyperthermic changes to 40°C have been shown to be associated with significant changes in OAE amplitudes (Khvoles et al., 1998; Seifert et al., 1998). Studies have shown that otoacoustic emission amplitude generally tends to decrease and ABR latency tends to increase when body temperature changes regardless if it is raised or lowered from normal body temperature (Ferber-Viart et al., 1995; Janssen et al., 1991; Khvoles et al., 1998; Markland et al., 1987; O'Brien, 1994; Seifert et al., 2001; Seifert et al., 1998; Taschenberger, & Manley, 1997); however, the further the temperature is from normal body temperature, the greater the change. This study was conducted to explore the effects of temperature on amplitude of DPOAEs and threshold of tone-burst ABRs.

Contrary to the quoted literature, the analysis of the DPOAE and ABR data obtained in this investigation indicated negative results. When all of the test subjects were considered, temperature essentially had no significant effect on DPOAE amplitude or ABR threshold. Therefore, based on this study it appears that taking body temperature before performing OAEs and ABRs does not need to be part of the standard test protocol. In addition, it is not necessary for normative data for DPOAE and ABR to be collected based on body temperature.

It is difficult to compare the results of the current study to other studies because most studies analyzed TEOAEs rather than DPOAEs however, physiologically it is doubtful that this would account for any differences. Note that due to the technique of its measurement, TEOAEs cannot provide information for frequencies higher than 4,000 to 6,000 Hz. Most hypothermic studies, far below the physiologic range, showed a decrease in TEOAE amplitude. The study by Seifert et al. (1998) conducted on humans undergoing heart surgery found that TEOAEs could not be recorded at a vesical temperature of 33.41ºC and a nasopharyngeal temperature of 30.16ºC. The TEOAEs reappeared after the patients were returned to normal body temperature. This was an obvious change; however the study did not report any significance testing for the 30 subjects used in the study. The hypothermic study conducted on guinea pigs showed significant findings in that TEOAEs disappeared at 27.3ºC, a temperature well outside the normal physiological range. Again, upon returning the animals to normal body temperature the TEOAEs returned. Khvoles et al. (1998) found a significant reduction in TEOAE amplitudes when rats' body temperature was decreased below 31ºC ($p < 0.01$).

Hyperthermic studies also found a decrease in TEOAE amplitude (Ferber-Viart et al., 1995; Khvoles et al., 1998). Ferber-Viart et al. found a clear reduction in TEOAE amplitude on humans when placed in a climatic chamber and temperature was raised to 38.5ºC; however their results were not statistically significant. The TEOAEs returned to normal amplitudes upon cooling to normal body temperature. Khvoles et al. showed a significant decrease in TEOAE amplitudes when rats' body temperature were raised to 40ºC ($p < 0.01$). In their study, two rats were heated to 41.5-42ºC. Death occurred in

one rat; in the other, the TEOAE disappeared and did not return upon cooling the rat to normal body temperature.

The study conducted by Khvoles et al. (1998) was one of the few to analyze TEOAEs as well as DPOAEs. The current study analyzed a larger range of DPOAE frequencies up to 15,984 Hz, whereas the study by Khvoles et al. (1998) analyzed DPOAE frequencies up to 8,000 Hz. For DPOAEs, the small differences in mean DPOAE amplitude in this current study were not statistically significant; however the direction of change was similar to the study by Khvoles et al. in that DPOAE amplitude decreased. Specifically, Khvoles et al. found a significant decrease in TEOAE amplitude ($p < 0.01$) and DPOAE ($p < 0.05$) amplitude at 2000 and 8000 Hz at 40°C. The temperature in this current study was raised to 40.5°C and the largest changes in DPOAE were more apparent at 40.5ºC; however the changes were not statistically significant.

In the current study, changes in DPOAE amplitude occurred first in the high frequencies followed by the mid-frequencies. One difference between this study and other studies is that DPOAE amplitude actually increased with an increase in temperature. These slight increases in DPOAE amplitude began to occur above 10,078 Hz for 38.5°C and 39.5°C. For 38.5°C and 39.5°C, DPOAE amplitude decreased in the mid-frequency range. The largest change or difference in DPOAE amplitude occurred above 3,562 Hz for 40.5°C. Specifically, the largest decrease in DPOAE amplitude was a difference of 3 dB SPL that occurred at 8,015 Hz for 40.5°C. Note, that all described trends were not statistically significant. It is also important to note that in this study as the higher body temperatures were reached (38.5°C and 39.5°C) the rats began to breathe

heavier and therefore were noisier. It is doubtful that the heavy breathing had an effect on the findings because all DPOAEs were at least 3 dB above the noise floor.

The study by Khvoles et al. (1998) showed that even an extreme decrease in temperature to 33°C, which is also well outside the normal physiological range, showed no significant changes in TEOAE or DPOAE amplitude. An additional decrease in temperature to 27°C showed a significant decrease in DPOAE amplitude at 2000 and 8000 Hz ($p < 0.01$).

It is possible that the larger sample size ($n = 16$) used in this current study accounted for the difference in significant results as compared with the study by Khvoles et al. (1998) as significance may have been caused by random fluctuations due to the small sample size ($n = 8$). This is supported by the findings in TG1, in which a statistically significant difference was seen for temperature. This difference was not evident when all 16 rats were considered together. Another speculation for differences in findings could be test time. However, other studies had an average test time of 30 to 108 minutes and the total test for each rat in the current study was five to six hours. This suggests that test time and amount of anesthesia had no effect on results either.

The negative results found in this current study may be the result of the highly controlled and lengthy protocol, which allowed for careful recordings and thorough analysis. It is possible that the protocol for other studies was more lax, therefore differences in their findings could potentially be erroneous.

The only significant main effect for temperature in this study occurred for DPOAEs in TG1. This significant finding is most likely an erroneous finding due to the small sample ($n = 8$). As mentioned previously, once the data were collapsed for both

trial groups (n = 16), the main effect for temperature was no longer significant. It is possible that a few rats in TG1 had a larger variability, therefore positively skewing the data. Upon examination of the raw data, it was obvious that four rats in TG1 had an usually high variability in their DPOAE data. Another possible explanation for this significant finding is the direction of temperature change. For example, the body temperature of rats in TG1 was slowly increased from the normal body temperature of 37.5º C, whereas the body temperature of the rats in TG2 was immediately increased to 40.5º C, then decreased back down to 37.5º C. The increased temperature change for TG1 is similar to the increased temperature protocol for the studies conducted by Ferber-Viart et al. (1995) and Khvoles et al. (1998), which had significant results for changes in DPOAE and TEOAE amplitudes. For the current study, all other DPOAE analyses showed a significant main effect for frequency (n = 16), but not for temperature or the interaction between temperature and frequency.

One speculation to account for changes in OAE amplitudes is that changes in body temperature may alter the middle ear pressure, thus affecting the amplitude of the OAE (Ferber-Viart et al., 1995; Seifert et al., 1998). To date, there have been a limited number of studies showing temperature effects on middle ear pressure and temperature effects of OAEs in humans.

Studies have shown frequency-specific effects of temperature on OAE amplitude. The hypothermic study by Seifert et al. (1998) showed TEOAE changes first in the mid-frequency range (1,600 to 2,000 Hz), then the high frequencies (4,000 Hz) and that higher frequency OAEs reappeared at a higher temperature than the middle-frequency OAEs. The two hyperthermic studies on TEOAEs showed a decrease in TEOAE

amplitude. The findings were significant in the 2,000-4,000 Hz spectral range at 40°C (Khvoles et al., 1998) however, the findings were not significant at 38.5°C in the 1,000 to 3,000 Hz spectral range for the study conducted by Ferber-Viart et al. (1995). The study by Khvoles et al. showed a significant decrease in DPOAE amplitude at 2000 and 8000 Hz at 40°C. Veuillet, Collet, and Morgon (1992) observed a similar response when looking at the affects of OAEs with both positive and negative pressure changes in the outer ear canal. The higher frequency emissions were not affected as much by pressure changes as the lower frequencies were. The greatest change in OAE amplitude occurred at –180 mmH_2O (Veuillet et al., 1992). Zheng, Ohyama, Hozawa, Wada, and Takasaka (1997) also showed frequency-specific characteristics when recording DPOAEs in a gerbil with the presence of both positive and negative middle ear pressure.

For negative pressure, DPOAE amplitudes decreased first in the low frequencies, then the high frequencies, and finally in the middle frequencies. Fewer effects on DPOAE amplitude were present at frequencies above 6,000 Hz for positive pressure (Zheng et al., 1997). These results are similar to the findings by Zhang and Abbas (1997) in their study of positive and negative middle ear pressure changes on OAEs in guinea pigs. This link is logical due to the fact that the stiffness component of the middle ear system mainly affects the transmission of low frequency sounds (Zhang & Abbas, 1997). Moore, Lippe, and Rubel (1995) stated that changes in middle ear pressure affect the "stiffness and damping of the tympanic membrane." The study by Zhang and Abbas (1997) also showed a significant change in high frequency OAEs when middle ear pressure was negative. Zhang and Abbas (1997) suggested that the pressure compresses the ossicles creating a mechanical contact, which introduces more mass into the middle

ear system, thus affecting the high frequencies. However, the study by Zhang and Abbas (1997) was done on guinea pigs, which makes it difficult to generalize to humans.

Other studies have shown that the middle ear status can impact the OAE being recorded (Gehr et al., 2004; Wada & Kobayashi, 1990; Zheng et al., 1997). Puria (2003) looked at several factors including middle ear impedance, stapes displacement, ear canal pressure, vestibule pressure, and reverse middle-ear pressure gain to obtain a better understanding of how the middle ear system changes the OAE from that originally created in the cochlea. Wada and Kobayashi (1990) stated that middle ear pressure affects the stiffness of the middle ear, which causes the movement of the middle ear system to decrease.

In addition, Voss and Shera (2004) stated that the forward and reverse middle ear transfer function can negatively impact the measurability of OAEs in the ear canal. Therefore, if middle ear pressure affects the middle ear system's ability to transmit vibrations, OAE measurement will be affected. Most studies determined that lower frequency emissions are affected more by pressure changes than higher frequencies are.

Although the current study did not monitor middle ear pressure, our findings contradict the theory of temperature potentially affecting middle ear pressure. Specifically, the frequency dependence of potential changes was opposite to observed middle ear pressure changes, i.e. changes in body temperature affected the higher frequencies first (above 10,078 Hz) with the largest decrease in DPOAE amplitude of 3 dB SPL that occurred at 8,015 Hz for 40.5°C; therefore any changes most likely are not due to changes in middle ear pressure. Slight changes in DPOAE amplitude are most likely due to the motility of the outer hair cells or that changes in body temperature

which changed the physical properties of the fluids in the cochlea (O'Brien; 1994). However to completely discredit the theory, temperature effects on middle ear pressure should be tested clinically on human adults and children. This study cannot be conducted for ethical reasons and any examination would need to be carried out by observation alone, eliminating the ability of researchers to assign cause and effect.

This study demonstrates that temperature essentially does not affect ABR thresholds. It is possible that any small differences in the ABR threshold occurred due to variability in equipment. It is difficult to compare this study to other studies because none of the other studies analyzed tone-burst, frequency-specific ABR thresholds. All other studies analyzed latency and amplitude of click-evoked ABRs. In addition, the regression analysis for each frequency and for each temperature showed no significant correlation between DPOAE amplitude and ABR thresholds. The slight changes in DPOAEs suggest that, anatomically, any potential changes must occur between the presentation of the stimuli up to the level of the cochlea, specifically at the level of the outer hair cells. The results of the initial body temperature of 37.5°C compared to the final temperature of 37.5°C for TG2 indicate that any decrease in DPOAE amplitude recovered upon return to normal body temperature ($p < 0.01$).

Future Studies

Previous studies looked at TEOAEs, which evaluate only up to 6,000 Hz and as this study shows, differences first appear in frequencies higher than 4,000 Hz. Therefore it is recommended that any future studies should be conducted using DPOAEs, which are designed to evaluate higher frequencies. Based on the literature, it would be interesting to conduct future research to continue to explore whether temperature affects middle ear

pressure although the results of the current study seem to contradict this idea. Ideally, future studies should be conducted on humans to determine the possibility of any interspecies difference in eliminating potential temperature effects on DPOAEs and ABRs. Additionally in this current study, changes in body temperature showed a potential frequency-dependent trend; therefore further studies should consider evaluating frequency-specific effects of body temperature on DPOAEs.

In conclusion, this study proposes no basis for suspected significant temperature effects for DPOAEs and ABRs. The final validation of these issues can be obtained only on humans. In many respects, the auditory system function of the rat is similar to the auditory system function of humans; therefore one would expect similar results.

APPENDIX

DATE: May 8, 2006

TO: Amanda N. Snyder

Department of Biological Sciences
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FROM: Institutional Animal Care and Use Committee

RE: IACUC PROTOCOL #SU0607RPR.01
"Temperature Effects on Otoacoustic Emissions and Auditory Brainstem Responses in Rats"

This is to certify that the Institutional Animal Care and Use Committee has reviewed your protocol and granted FULL APPROVAL. Your approval date for this protocol is May 2, 2006.

Your protocol is approved for a period of 3 years; an annual report must be submitted to the IACUC one month before each anniversary of the protocol. Please note that your protocol will expire on May 2, 2009. If you need to extend the protocol beyond this date, you must submit an Animal Care and Use Form at least 3 months prior to the expiration.

If you have any questions, please do not hesitate to contact the IACUC Chair by email (detolla@vetmed.umaryland.edu) or by phone (at 410-706-8537).

Louis J. DeTolla, VMD, PhD
Chairman, IACUC

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