

IMPACT OF NEGATIVE MIDDLE EAR PRESSURE ON DISTORTION PRODUCT  
OTOACOUSTIC EMISSIONS

by

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## Abstract

### IMPACT OF NEGATIVE MIDDLE EAR PRESSURE ON DISTORTION PRODUCT

#### OTOACOUSTIC EMISSIONS

by

SUZANNE THOMPSON

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Negative middle ear pressure (NMEP) is a disorder of the middle ear in which the tympanic membrane is retracted and pulled inward. Structures of the middle ear are compressed, and there is an increase in middle ear impedance. NMEP can modify estimates of auditory function such as Distortion Product Otoacoustic Emissions (DPOAE), which are generated in the cochlea when two primary tones ( $f_1$ ,  $f_2$  with  $f_2 > f_1$ ) are presented simultaneously to the ear and detected in the ear canal. Inter-modulation between primary tones produces distortion products at predictable frequencies not present in the original signal. The  $2f_1 - f_2$  DPOAEs measured in the ear canal are a composite of two components both having the same frequency, but originating via different mechanisms from different cochlear locations and having different phase properties. One component is generated by the nonlinear response of the basilar membrane (BM) to the overlapping traveling waves generated by the primary tones. The other linear reflection component arises when a portion of energy generated at the overlap region travels to its own characteristic place on the BM, where it is partially reflected by pre-existing perturbations.

NMEP was voluntarily induced when subjects performed the Toynbee maneuver (subjects to pinch their noses and swallow until a sensation of fullness is felt). Logarithmic frequency sweeping primaries (primary levels  $L_1/L_2 = 65/65, 70/70, 75/75$  dB SPL) producing

$2f_1-f_2$  from 320-2560 Hz were presented during interleaved trials normal middle ear pressure and subject-induced NMEP. The sweeping primary procedure provided detailed information about DPOAE level as a function of frequency and permitted estimates of the composite DPOAE and the extraction of the two major components. Extraction of the levels and phases of the sweeping primaries provided information about the changes in middle ear function.

At the primary levels tested, the nonlinear distortion component dominated the composite DPOAE. NMEP reduced the levels of the composite DPOAE, generator, and reflection components from 1060 Hz to 3537 Hz. Separation of the components reduced variability and permitted more reliable evaluation of the impact on NMEP on DPOAE.

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## **Chapter 1. Introduction**

The Joint Committee on Infant Hearing (JCIH) screening's position statement endorses the goal of universal detection of hearing loss in infants and encourages ongoing research with the goal of improving screening and intervention methods in a cost-effective manner ("Year 2007 position statement: Principles and guidelines for early hearing detection and intervention programs," 2007). Early detection of hearing loss and appropriate intervention are important because research indicates that early-identified infants (by 6 months of age) who receive appropriate services perform significantly better than their later identified peers (Yoshinaga-Itano, Sedey, Coulter, & Mehl, 1998). Early identified infants demonstrate better vocabulary development, improved receptive and expressive language skills, syntax, speech production, and better overall social-emotional development than later identified infants ("Year 2007 position statement: Principles and guidelines for early hearing detection and intervention programs," 2007).

Sound-evoked otoacoustic emissions (OAEs) are a tool commonly used in newborn hearing screening programs because they are an objective and non-invasive test of inner ear function (e.g., Gorga, Preissler, Simmons, Walker, & Hoover, 2001). During OAE testing, a small probe assembly with one or two speakers and sensitive microphone is placed in the ear canal. A speaker sends stimuli into the ear canal where it travels through the middle ear to the cochlea. Classically, in the cochlea, evoked otoacoustic emissions (EOAEs) are categorized as energy generated as the by-product of the normal cochlear amplification process. Some energy generated in the cochlea travels outward and is recorded by the microphone in the ear canal.

The introduction of Universal Newborn Hearing Screening (UNHS) has greatly improved early-detection and intervention of hearing loss in the newborn population ("Year 2007 position

statement: Principles and guidelines for early hearing detection and intervention programs," 2007). However, the current UNHS referral rate is at 2%, which is approximately 10 times greater than the prevalence of cochlear hearing loss (approximately 1-3 out of 1000 births) (Sanford, Keefe, Liu, Fitzpatrick, McCreery, & Lewis, 2009). Many of the referrals are due to transient external or middle ear conditions that obstruct the sound stimuli pathway (Spivak, Sokol, Auerbach, & Gershkovich, 2009) and inhibit the travel of the primary tones used to evoke the OAE and the reverse travel of the emission itself.

There are two problems with the current hearing screening protocol. First, the newborn's middle ear is not evaluated. This is because there are few valid and reliable tests of middle ear function for newborns (for review, see Hunter, Tubaugh, & Jackson, 2008). Tympanometry, including multi-frequency tympanometry, cannot be performed because the infant's ear canal is too flaccid to be pressurized (Holte, Margolis, & Cavanaugh, 1991). Second, DPOAEs are only tested at discrete frequencies in typical clinical paradigms. For example, the Natus Echo-screen Hearing Screener used in many hospitals collects DPOAEs at four frequencies (2000, 2500, 3200 and 4000 Hz). Lower-level DPOAEs, possibly caused by increased impedance due to fluid in the middle ear or NMEP, may be in the noise floor and may not be detected at these frequencies. Therefore, the fail would not be because of cochlear pathology, but because of a middle ear problem for which there is no standardized method of evaluation (Hunter et al., 2008). Research into acoustic reflectance and DPOAE measures during NMEP may improve understanding and use of current clinical techniques.

### **1.1. Evoked otoacoustic emissions**

EOAEs occur in response to sound stimulation and have been classically categorized into three subtypes based on the type of stimuli used to evoke them (for review see Shera, 2004).

Transient evoked otoacoustic emissions (TEOAEs) are elicited by transient stimuli such as clicks, tone-bursts or chirps (Kemp, 1978). Stimulus frequency otoacoustic emissions (SFOAEs) are elicited by a single continuous pure tone. Distortion product otoacoustic emissions (DPOAEs) are generated when two external (primary) tones close in frequency ( $f_1$  and  $f_2$  with  $f_2 > f_1$ ) are presented simultaneously in the ear canal. Inter-modulation between the traveling waves associated with the two primary tones (e.g., Kemp, 1979) produces distortion products at predictable frequencies not present in the original signal, for example  $2f_1 - f_2$ . Each type of EOAE is present in healthy cochleae and reduced in level, or absent, in the presence of cochlear pathology (e.g., Lonsbury-Martin & Whitehead, 1991).

EOAEs can be classified based on the mechanisms responsible for their generation (Shera & Guinan, 1999; cf. Shera, 2004). Linear reflection is believed to be responsible for the generation of TEOAEs, SFOAEs at low-levels, and the linear reflection component of the DPOAE (e.g., Kalluri & Shera, 2001, 2007; Shera, 2004; Shera & Guinan, 1999). DPOAEs measured in the ear canal are due to an interaction of at least two components from different regions of the cochlea, the component generated by linear reflection and a component generated by nonlinear distortion (e.g., Shera, 2004; Shera & Guinan, 1999; Talmadge, Long, Tubis, & Dhar, 1999). The nonlinear distortion component, also known as the ‘generator’ or ‘wave-fixed’ component, originates near the  $f_2$  region and is a result of the nonlinear response of the basilar membrane to the overlapping traveling waves generated by the two primary tones  $f_1$  and  $f_2$  (e.g., Mauermann, Uppenkamp, van Hengel, & Kollmeier, 1999a; Shera & Guinan, 1999; Talmadge et al., 1999). Generator component phase is relatively constant with changes in frequency because the region of maximum overlap where the generator component originates is at the peak of the higher-frequency traveling wave, and the wavelength to the peak of all traveling waves on the

cochlear partition is thought to be approximately the same. Consequently, the reverse traveling wave of generator component has little phase accumulation and is said to be wave fixed (Kemp, 1986).

When the DPOAE is lower in frequency than the primary frequencies, such as the  $2f_1-f_2$ , the linear reflection component of the DPOAE arises when a portion of the energy generated at the overlap region travels apically along the cochlear partition to its own characteristic place on the basilar membrane (i.e.,  $2f_1-f_2$ ). There it is partially reflected back towards the stapes by pre-existing mechanical perturbations on the membrane (Kalluri & Shera, 2001, 2007; Mauermann et al., 1999a; Mauermann, Uppenkamp, van Hengel, & Kollmeier, 1999b; Shera, 2004; Shera & Guinan, 1999; Talmadge et al., 1999). The phase of the reflection component varies rapidly with changes in primary frequency because the irregularities on the cochlear partition are randomly distributed (e.g., Shera & Guinan, 1999; Talmadge et al., 1999) and the phase of the basilar membrane response varies rapidly with frequency near its characteristic place on the basilar membrane. However, the reflection region where the wave peaks as a function of frequency could be considered as fixed in place. Therefore, the reflection component is also known as the 'place-fixed' component. The reflection component must also travel further along the basilar membrane and has a longer latency than the generator component because its traveling wave slows as it nears its best frequency. The phase of the reflection component depends on the combined effects of its phase at generation and travel time.

In a healthy ear, energy from the two components interacts and yields the DPOAE level and phase recorded in the ear canal (for review, Shera & Guinan, 1999). DPOAE level fluctuations are a consequence of variations in the vector summation of the two components with frequency (e.g., Dhar & Shaffer, 2004; Long, Talmadge, & Lee, 2008; Mauermann et al., 1999a;

Shera & Guinan, 1999; Stover, Neely, & Gorga, 1996; Talmadge et al., 1999); both component have the frequency  $2f_1-f_2$  but originate from different cochlear locations via different mechanisms of generation (e.g., Shera & Guinan, 1999). The two components, each with their own phase, sum either constructively or destructively and are measured as the composite DPOAE in the ear canal (e.g., Shera & Guinan, 1999; Talmadge et al., 1999). If the generator and reflection components are summed while in phase, then DPOAE level reaches a maximum, or peak, in the composite DPOAE. However, if the two components are summed out of phase, they partially cancel one another and produce a level minimum, or dip, in composite DPOAE. Due to the interaction between the two components, DPOAE levels can vary as much as 20 dB in healthy cochleae with small changes in frequency, and this variability will affect the validity of the DPOAEs as an indicator of cochlear health (e.g., Mauermann et al., 1999b; Talmadge et al., 1999). Additionally, DPOAE level and phase are highly dependent on stimulus frequency separation (Brown & Gaskill, 1990; Gaskill & Brown, 1990) and primary level (He & Schmiedt, 1997; Kummer, Janssen, & Arnold, 1998).

### **1.2. Clinical importance of composite DPOAE**

Most clinicians that measure DPOAEs do so at discrete frequencies. The clinicians are often unaware of the effect that the composite DPOAE can have on their measurements because the data is not measured with high frequency resolution. Therefore, a clinician does not know if a measure was made at a peak or valley in the DPOAE. An infant may fail at a discrete frequency if the measure falls in a valley of the fine structure, close to the noise floor. This negatively affects the validity of the test because the infant actually does have easily measureable DPOAEs, if only the correct test frequencies are chosen.

Clinical and research utility of DPOAEs are improved when the DPOAEs are evaluated with high frequency resolution (e.g., Kummer et al., 1998; Mauermann et al., 1999b; Shera, 2004; Shera & Guinan, 1999). When the composite DPOAE was evaluated in 8 humans with different configurations of mild-moderate hearing losses (Mauermann et al., 1999b) the depth of the DPOAE was influenced by which component fell in the region of cochlear damage. If the hearing loss was in a region close to the reflection source, but the primary stimuli were located in a region of near-normal hearing, then the distortion product  $2f_1-f_2$  was still at normal levels, but the fine structure was gone (Mauermann et al., 1999b). However, if the reflection component fell in a region of normal hearing and the primary stimuli were in a region of mild hearing loss, the overall level was reduced, but the fine structure was preserved (Mauermann et al., 1999b).

Separating the generator and reflection components from the composite DPOAE offers additional advantages and has the potential to provide information about cochlear nonlinearities and the cochlear amplifier (e.g., Long et al., 2008; Mauermann & Kollmeier, 2004; Mauermann et al., 1999b; Shera & Guinan, 1999). Attempts have been made to use DPOAE input/output (I/O) functions to predict audiometric thresholds (Boege & Janssen, 2002; Kummer et al., 1998) and determine characteristics of basilar membrane compression (e.g., Neely, Gorga, & Dorn, 2003). When the components were not separated, the error was always large. The large error in predicted behavioral thresholds based on DPOAE I/O functions was lessened when the components were separated and the reflection source was removed from the I/O function (e.g., Mauermann & Kollmeier, 2004).

Separation of the components was also useful when studying efferent effects on DPOAE level and phase (Henin, Thompson, Abdelrazeq, & Long, 2011). Efferent fibers synapse directly onto outer hair cells and during contralateral acoustic stimulation they modify outer hair

cell amplification. DPOAEs, which are a by-product of outer hair cell amplification, will also be modified during efferent activation. Efferent effects on the composite DPOAE were difficult to interpret because both suppression and enhancement of DPOAE level were observed during contralateral stimulation (e.g., Henin et al., 2011). However, more consistent and easier to interpret changes, mostly suppression of emission level, were observed in the generator and reflection components after the DPOAE components were separated (Henin et al., 2011).

### **1.3. Middle ear**

The middle ear is important for sound transduction from the low-impedance air to the high impedance fluid of the cochlea. The outer and middle ears are able to partially match the sound conductive properties of air and fluid by increasing the sound pressure that reaches the inner ear. Classical understanding of middle ear function is that the major mechanism of this transformation is the area difference between the TM and the stapes footplate; for humans, the area of the TM is approximately 20 times larger than that of the footplate (Weaver & Lawrence, 1954; reviewed in Merchant & Rosowski, 2003). The sound pressure collected over the large area of the TM is concentrated on the much smaller area of oval window of the cochlea where the stapes footplate rests. There is also a small difference in lengths of 2 middle ear bones, the malleus and incus (1.3:1). Due to this difference in length, the rotation of the malleus and incus arms around the axis of rotation acts as a lever resulting in an additional approximately 2 dB sound pressure increase (Kirikae, 1960).

Theoretical calculation of the middle ear sound pressure gain in humans is approximately 28 dB (26 dB TM area ratio and 2 dB ossicular lever). However, measurements of actual middle ear sound pressure in human middle ears demonstrate a frequency-dependent pressure gain with a maximum gain of only about 23 dB near 1200 Hz (Aibara, Welsh, Puria, & Goode,

2001). The gain in humans is represented as bandpass in nature rising systematically up to 1000 Hz before peaking and then systematically rolling off at frequencies above 3000 Hz (Aibara et al., 2001; Puria, 2003).

The discrepancy between the pressure increase predicted by a simple middle ear model and the observed increase is due to several non-ideal conditions within the middle ear system. Unlike a simple model, the TM does not move as one body (reviewed in Merchant & Rosowski, 2003; Rosowski, 2003). The pattern of vibration depends on the frequency of the sound (Decraemer, Khanna, & Funnell, 1989; Tonndorf & Khanna, 1972). Above 1000 Hz the pattern of vibration becomes more complicated as the TM no longer vibrates as a whole. This leads to phase differences across the membrane. The effect of the wave pattern on the TM to sound transmission is not well understood. Consequently, not all the sound pressure moves the TM and ossicles. Some sound pressure is therefore lost before it reaches the cochlea. The air in the middle ear and the structures of the middle ear also limit the pressure increase, and there is a loss due to movement in the ossicular system particularly around 1000 and 2000 Hz. This loss has been associated with movement of the rotational axis of the ossicles and the ossicular joints, which reduces the motion (Guinan & Peake, 1967).

Middle ear pressure gain in humans appears to decrease systematically as a function of frequency (e.g., Aibara et al., 2001; Puria, 2003). The middle ear pressure gain, as defined as ear canal sound pressure to cochlear vestibule pressure gain, in 12 fresh human temporal bones reached a magnitude of 23.5 dB at 1200 Hz with a slope of about 6 dB per octave from 100 to 1200 Hz and -6 dB per octave beyond 1200 Hz (Aibara et al., 2001). When an additional tube was used to reduce the vibration produced by the inner ear sound source in human temporal bones (Puria, 2003) there was a maximum pressure gain (five-ear-average) of 18 dB at 900 Hz

(Puria, 2003). Below 800 Hz the slope was approximately 10.4 dB per octave and between 1000 and 6000 Hz the slope averaged -7.2 dB per octave (Puria, 2003). The differences were probably the result of the additional tube, which likely altered the cochlear input impedance, but did not affect the ability to make middle ear estimates (Puria, 2003).

The middle ear is not as efficient in the reverse direction. There is a reverse pressure loss in the human middle ear for sounds generated in the cochlea. The reverse pressure change, the ratio of ear canal pressure to inner ear pressure, in humans is generally band-pass in shape and reaches a maximum of about -30 dB at 1400 Hz (Puria, 2003). Below 1200 Hz the reverse pressure decreases with a slope of approximately -4.1 dB per octave. Above 1400 Hz the reverse pressure decreases approximately -15.5 dB per octave as more pressure is reflected back to the cochlea (Puria, 2003).

Forward and reverse transmission through the middle ear have also been measured in gerbil middle ears by comparing pressure in the scala vestibuli at the stapes to ear canal pressure near the TM (e.g., Dong & Olson, 2006). In gerbils, the middle ear forward pressure gain is approximately 20 to 25 dB and essentially flat with frequency from 2000 to 30,000 Hz (Dong & Olson, 2006). The reverse pressure loss is also relatively flat with frequency and has a value of about -30 to -40 dB (Dong & Olson, 2006). The pressure decrease over the same frequency range is about 10 to 15 dB more for reverse travel than the forward transmission pressure increase (Dong & Olson, 2006).

The differences in gain between forward and reverse transmission in humans (Puria, 2003, Puria & Rosowski, 1996) and gerbils (Dong & Olson, 2006; Dong, Decraemer, de La Rochefoucauld & Olson, 2010) are due to TM motion, the impedance of the TM, which is affected by outer ear canal volume, and ossicular motion. Due to the negative reverse

transmission process, portions of the energy generated by the distortion product will be reflected back into the cochlea at the stapes footplate (Puria, 2003). Puria (2003) estimates that more than  $\frac{2}{3}$  of distortion product energy below 1000 Hz in human adults is reflected back into the inner ear (Puria, 2003). Between 1000 and 4000 Hz the least amount of energy is reflected back from the stapes. This is also the frequency range at which the middle ear most efficiently transfers energy in the forward direction, and is the frequency region that provides robust OAE measures in adults. Beyond 4000 Hz the amount of energy reflected off the stapes generally increases (Puria, 2003).

All OAE testing, including fine and discrete measures, depend on the health of the middle ear. Therefore, to improve OAE measurements, it is important to understand the mechanical properties and impedance of the middle ear. Impedance is the total opposition of motion offered by a system and is a complex combination of three components: resistance, mass reactance, and compliant reactance (for review see, Wiley & Stoppenback, 2001). Resistance is the opposition to energy flow and is provided by the friction of a system. It is always present, in phase with the application of sound pressure and independent of frequency. Resistance dissipates energy in the form of heat. Reactance is the energy storage component of impedance and is out of phase with the application of sound pressure. There are two types of reactance including mass and compliant reactance. Both contribute to the total impedance offered by the system. Mass reactance is most influential during high frequency sound transmission, and compliant reactance is dominant during low frequency transmission.

Friction in the middle ear system results from the tympanic membrane (TM), tendons and ligaments, the viscosity of the perilymph, and the mucous lining of the middle ear cavity (for review see Wiley & Stoppenback, 2001). The pars flaccida of the TM, the ossicles, and the

perilymph in the cochlea contribute to the mass reactance of the middle ear system. The stiffness elements of the middle ear system include the ligaments, tendons, the TM, and the air enclosed in the ear canal and middle ear spaces.

The middle ear is a complex mechanical system, and modeling the middle ear can simplify analysis and help one understand how it functions. One of the simplest models is the lumped element model. The lumped element model simulates the middle ear as a combination of idealized mechanical elements. Results from the lumped element middle ear model show that the healthy adult middle ear is stiffness dominated below 800 Hz, with a resonance between 800 and 1200 Hz (when the stiffness and mass reactance cancel out and total impedance is determined by resistance) and mass loaded at frequencies above 1200 Hz (for review see Fowler & Shanks, 2001).

Pathologies of the middle ear system may change the mechanical and acoustic characteristics of the middle ear transfer function in complex ways. For example, increased stiffness may result from otitis media with effusion. According to the lumped element model, stiffness increases the resonant frequency of the system. Mass reactance increases due to pathology such as ossicular discontinuity. In this case, the resonant frequency of the middle ear decreases (reviewed in Fowler & Shanks, 2001). This simple model is less than perfect, however, because the middle ear may have more than one resonance frequency. If that is the case, middle ear pathologies will have more complex effects on the multiple middle ear resonances, which cannot be modeled using the lumped element model, and more complex middle-ear models are needed.

#### **1.4. Otoacoustic emissions and middle ear static pressure**

An increase in middle-ear impedance will affect both the forward travel of stimuli used to evoke the emission and the reverse travel of the emission. Pathology of the middle ear, including a perforated or retracted TM and fluid in the middle ear space, will change the impedance of the middle ear system and may reduce OAE levels or change OAE measurements. This is of particular concern for newborn hearing screening programs because test validity is extremely important.

Negative middle ear pressure (NMEP) and fluid in the middle ear space are common conductive pathologies, which are mainly attributed to Eustachian tube dysfunction. The Eustachian tube connects the middle ear space to the nasopharynx, and, when working properly, balances the pressure in the middle ear space with the pressure in the environment. It also drains debris from the middle ear space. Dysfunction of the Eustachian tube prevents the normal processes of middle-ear aeration and pressure balancing, which leads to reduced or negative pressure in the middle ear space. NMEP is common in young people because their Eustachian tubes tend to be narrower and more horizontal and thus do not drain debris from the middle ear as well as adult Eustachian tubes, which are larger and on a more vertical plane (e.g., Fria, Cantekin, & Eichler, 1985). Consequently, young children are more susceptible to ear infections and fluid in their middle ear space caused by congestion.

NMEP may lead to little change in pure tone thresholds or minimal mild hearing loss depending on the extent of the pressure change (e.g., Sun & Shaver, 2009). In most clinical settings  $\pm 100$  daPa, as measured by tympanometry, is still considered a normal middle ear pressure (for review see Margolis & Hunter, 1999). However,  $\pm 100$  daPa, and less, may influence OAE levels (e.g., Marshall, Heller, & Westhusin, 1997).

Both positive and negative pressure changes in the ear canal or middle ear space will affect the impedance properties of the middle ear. NMEP retracts the TM, compresses the ossicular chain, and increases stiffness of the middle ear apparatus, thereby increasing the stiffness reactance of the system. NMEP is predicted to decrease transmission of low-frequency sounds more than mid- or high- frequency sounds. However, NMEP will still have an effect on high-frequency transmission because it changes the position of the ossicular chain. NMEP may increase the flexure of the chain and the ossicular chain's contact with other structures such as the wall of the middle ear cavity or the oval window (Zhang & Abbas, 1997). These changes will increase the mass reactance of the system and ultimately reduce transmission at higher frequencies in addition to low and mid frequencies (Zhang & Abbas, 1997).

Positive and negative changes in middle ear pressure affect OAE measurements in a frequency-specific manner (van Dijk, Maat, & de Kleine, 2010). However, it is difficult to do controlled research on the effects of middle ear pressure because it is difficult to consistently manipulate an individual's middle ear pressure for a period of time long enough to obtain OAE measures. Different methods have been used with varying degrees of effectiveness.

Two methods used in research produce a pressure difference between the outer ear and the middle ear, but do not actually manipulate middle ear pressure. The first method alters atmospheric pressure in a pressure chamber in which the subject is seated (Konradsson, Svensson, Carlborg, & Grenner, 1999; Richter, Hauser, & Lohle, 1994). This is similar to going up or down in an airplane. The second method changes the air pressure in the ear canal using a probe placed in the subject's ear (Osterhammel, Nielsen, & Rasmussen, 1993; Robinson & Haughton, 1991; Plinkert, Bootz, & Vossieck, 1994). Both methods have similar effects (Konradsson et al., 1999; Osterhammel et al., 1993; Plinkert et al., 1994; Richter et al., 1994;

Robinson & Haughton, 1991). Introduction of positive pressure to the ear canal at ambient pressure pushes the TM inward. An introduction of negative pressure to the ear canal pulls the TM outward, similar to when there is a positive pressure in the middle ear space. Though changing the ambient pressure of a room or manipulating the pressure in the ear canal do create a pressure difference between the outer and middle ears, they are not optimal techniques for estimating the effects of middle ear pressure on OAEs because the methods do not change the actual pressure in the middle ear space.

Nonetheless, frequency-dependent changes in EOAE level were found when the pressure in the ear canal was increased or decreased, but the introduction of positive pressure to the ear canal, which displaced the TM inward, and is most similar to Eustachian tube dysfunction associated with NMEP, had more of an effect than the introduction of negative pressure in the ear canal (Plinkert et al., 1994; Robinson & Haughton, 1991). The largest mean reductions of EOAE level occurred at the lowest frequencies tested, and EOAE level reductions became progressively less apparent at frequencies beyond 3000 Hz (Konradsson et al., 1999; Osterhammel et al., 1993; Richter et al., 1994). Finally, the most extreme pressure changes had the greatest effect (Koike & Wetmore, 1999; Osterhammel et al., 1993; Robinson & Haughton, 1991) though even small pressure differences across the TM (-50 daPa) reduced EOAE levels (Konradsson et al., 1999; Osterhammel et al., 1993; Richter et al., 1994).

Correcting for existing middle ear pressure differences by modifying the pressure in the outer ear corrects the position of the TM but may not fully correct all the changes in the middle ear. However, this procedure does permit evaluation of matched and mismatched pressures in the same subject and does have clinical relevance. Compensating for NMEP by introducing an equal amount of negative pressure in the ear canal so that the pressures on both sides of the TM

are equal increased DPOAE and TEOAE levels to values close to those obtained at ambient pressure (e.g., Hof, Dijk, Chenault, & Anteunis, 2005; Trine, Hirsch, & Margolis, 1993). Also, measuring TEOAEs at compensated middle ear pressures resulted in higher TEOAE pass rates (Hof et al., 2005; Trine et al., 1993). TEOAE levels increased more for lower frequency bands than higher frequency bands (Hof et al., 1997; Trine et al., 1993), and un-equalized middle ear pressures reduced low-frequency emissions more than high-frequency emissions (Trine et al., 1993).

The previous procedures modified outer ear pressure and created a discrepancy between the outer and middle ear pressures that either pushed or pulled the TM out of place, thus producing hearing loss and impedance changes. These methods work, but they might not fully simulate changes in middle ear pressure because only the pressure in the ear canal is being changed. Procedures that do modify middle ear pressure include the Toynbee and Valsalva maneuvers.

The Toynbee and Valsalva maneuvers can be used to voluntarily induce negative or positive middle ear pressure. The subject is asked to pinch his or her nose and close their mouth while swallowing (for the Toynbee maneuver, which decreases middle ear pressure), or blowing (for the Valsalva maneuver, which increases middle ear pressure), until a sensation of fullness is felt in ears. The subject is then asked to refrain from swallowing which would release the pressure. Subjects may have difficulty performing the Toynbee; in one study only 67% of ears were able to generate a negative shift (-15 to -99 daPa) (Riedel, Wiley, & Block, 1987). Inability to perform the Toynbee and variability in negativity may result from many factors, which include motivation, fatigue, failure to understand instructions, and variations in middle ear and Eustachian tube physiology (Riedel et al., 1987). Despite the challenges associated with

Toynbee and Valsalva maneuvers, they are relatively easy methods to evaluate middle ear pressure and are the only procedures that directly modify middle ear pressure. The procedures have few drawbacks, provided the subject can induce the pressure change.

Sun and Shaver (2009) evaluated the effects of induced NMEP using the Toynbee maneuver in 16 normal hearing adults (-40 to -420 daPa) at 9  $f_2$  frequencies used to generate  $2f_1$ - $f_2$  DPOAE (600-8000 Hz) and then again with higher frequency resolution. There was a mean reduction in DPOAE level of -4 to -6 dB for NMEP (-100 daPa and better) in low to mid frequencies (Sun & Shaver, 2009). Increasing the NMEP (-160 daPa and beyond) resulted in greater reduction of DPOAE level (e.g., -10 to -12 dB) (Sun & Shaver, 2009). NMEP was found to attenuate DPOAEs more at low frequencies (<1000 Hz) than high frequencies (Sun & Shaver, 2009).

The patterns of results when the pressure across the TM was changed were similar to those when the middle ear pressure was manipulated using the Toynbee maneuver. NMEP, or the induction of a positive pressure to the ear canal, pushes the TM inward and has a greater effect on EOAE measurements than positive pressure introduced to the middle ear space or negative pressure in the ear canal, which pulls the TM outward (e.g., Konradsson et al., 1999; Plinkert et al., 1994; Richter et al., 1994; Hauser, Probst, & Harris, 1993). Greater changes in EOAE magnitudes were observed for low-frequency EOAEs than high-frequency EOAEs (e.g., Sun & Shaver, 2009; Hauser et al., 1993) and more extreme pressure changes had greater effects on EOAEs (Sun & Shaver, 2009; Owens, McCoy, Lonsbury-Martin, & Martin, 1993). Finally, a common finding was variability in OAE level at the higher frequencies (e.g., Hauser et al., 1993; Marshall et al., 1997; Osterhammel et al., 1993; Robinson & Haughton, 1991). Large inter-subject variability was also noted (e.g., Hauser et al., 1993; Osterhammel et al., 1993).

### **1.5. Composite DPOAE and middle ear static pressure**

NMEP affects all types of OAEs because it modifies the effective levels of the stimuli used to evoke the OAE and it makes it difficult for the OAE to leave the cochlea. NMEP increases the impedance of the middle ear system in a manner similar to that produced by middle-ear muscle contraction evoked by high sound pressure levels (e.g., 80 dB SPL) (Henin & Long, 2012). Henin et al., (2012) analyzed DPOAE primary level and phase changes for two primaries, L40 and L50 dB SPL, at two contralateral acoustic stimulation levels, 40 and 80 dB SPL. Contralateral acoustic stimulation level 40 dB SPL was chosen because the middle ear muscle was not predicted to contract at this level, and 80 dB SPL was chosen because the middle ear muscle reflex was predicted to contract at this higher level. During contralateral acoustic stimulation 40 dB SPL, there was no change in primary  $f_1$  level or phase for all subjects. However, at contralateral acoustic stimulation level of 80 dB SPL, there were consistent changes in DPOAE primary level and phase. There was an increase in reflected energy for low frequencies (420-800 Hz) followed by a decrease in reflected energy (800-1500 Hz) (Henin et al., 2011). The changes were consistent with an increase in middle-ear resonance during middle ear muscle contraction and were similar to hypothesized changes in wideband reflectance estimates during middle ear muscle contraction (Feeney & Keefe, 1999). Similar changes in DPOAE primary level and phase are hypothesized during NMEP because it pulls the TM inward, increases the resonance of the ear canal, and increases the impedance of the middle ear system. Thus, an increase in primary reflected energy followed by a decrease in primary reflected energy is predicted during NMEP.

The effects of NMEP on composite DPOAE and its components level or phase have not been systematically examined. The composite DPOAE, when swept across frequency, is

hypothesized to be a more sensitive indicator of middle ear function than measures of EOAEs obtained at isolated frequencies. Sweeps will give a more detailed picture of the DPOAE changes with frequency.

Furthermore, since NMEP alters the transmission properties of the middle ear, and energy generated in the cochlea may have difficulty exiting the middle ear, more energy will be reflected off the stapes footplate of the middle ear back into the cochlea. The energy reflected back into the cochlea travels to its characteristic frequency on the basilar membrane where it will be re-reflected basally toward the middle ear. Multiple reflections of energy between the oval window and the characteristic frequency place may result and interact to modify the emission (van Dijk, et al., 2010). During NMEP, it is predicted that when the original and reflected energy are out-of-phase there may be deep minima in the DPOAE fine structure due to differences in phase of the components near the DPOAE region. When the original and reflected energy are in-phase near the reflection region a larger than usual DPOAE is expected. When reflections are large, the phase properties of the DPOAE may be different, which may increase the width of the fine structure. Wider spacing may also occur at high stimulus levels (L65, L70, L75 dB SPL) during NMEP because as energy gets larger in the cochlea at the characteristic frequency place, the properties of the reflection are hypothesized to change (Talmadge, Tubis, Long, & Tong, 2000; Long, Henin, & Thompson, 2011). Consequently, the DPOAE fine structure of persons with large internal reflection components and middle ear pathology is predicted to differ from that seen in persons with normal hearing.

## **1.6. Summary**

Early identification of hearing loss and advances in treatment methods improve the quality of life for many individuals. However, transient middle ear pathologies affect the

validity of OAEs as an objective and non-invasive tool for evaluating cochlear function. Most reviewed research into the effects of NMEP on EOAEs utilized coarse frequency resolution and did not evaluate the DPOAE fine structure. Therefore, it is unclear if the DPOAE measurements were collected in maxima or minima. None of the researchers separated the components nor did they know which component was dominant. Also, none looked at composite DPOAE or component phase changes.

This research evaluated artificially induced changes in middle ear pressure in persons with normal middle ears and measured the effects of NMEP in the composite DPOAE and its components. The Toynbee method was used to induce NMEP because it is an established model for producing clinical changes in middle ear pressure. The Toynbee is easy to perform and has thus far demonstrated consistent changes in middle ear pressure for a time period sufficient to obtain DPOAE measurements using our lab's OAE technique.

The goal of this study was to determine if changes in composite DPOAE occurred during NMEP and if these effects might be used to identify conductive pathologies. DPOAE measurements will be collected at normal pressure and compared with those obtained at NMEP. Furthermore because the level and phase of the primaries at the entrance to the ear canal will be modified when middle ear transmission is modified (Henin et al., 2011; Guinan, 2006), it was anticipated that DPOAE primary level and phase information can be used to estimate changes in middle ear transmission during data collection.

### **1.6.1. Hypotheses**

1. NMEP will increase stiffness reactance and this will increase the frequency of the ear canal resonance, which will result in changes in the level and phase of the primary tones  $f_1$  and  $f_2$  used to evoke the DPOAE. Comparison of the primaries between normal

pressure and NMEP will allow for trial-by-trial confirmation of subject-induced NMEP prior to composite and component DPOAE analysis.

2. NMEP increases the impedance of the middle ear system and will affect the forward travel of stimuli (primary tones  $f_1$  and  $f_2$ ) used to evoke the emission. Depending on the magnitude of the NMEP, composite DPOAEs generated by low-frequency primaries will be reduced in level. Low frequencies will be more affected than high frequencies.
3. The change in middle ear impedance will also affect the reverse transmission of the OAEs from the cochlea. The change in impedance will result in re-reflections back into the cochlea. Consequently, DPOAE fine structure measurements collected during NMEP will differ from those collected at normal pressure. In comparison to normal cochlear function, DPOAE fine structure for those with large reflection components and NMEP may have deeper peaks and valleys and wider frequency spacing between maxima especially at high primary levels.
4. Since the two major DPOAE components depend on different cochlear processes, we anticipate that separating the components will provide additional information about the effects of NMEP on OAE generation. NMEP effects will be clearer for the generator component because variability will be reduced. The magnitude and phase of reflection components are hypothesized to be less predictable.
5. The difference in composite DPOAE, generator, and reflection component level as well as phase under different middle ear pressures may permit separation of normal middle ear pressure from negative pressure in research and clinical applications.

## **Chapter 2. Method**

### **2.1. Subjects**

Twenty-six subjects were recruited. Otoloscopic examinations of the ear canal were performed to reveal that there was little accumulation of cerumen and the TM was intact. Audiometric thresholds were obtained at octave frequencies from 125 Hz to 8000 Hz to ensure air conduction thresholds better than 15 dB HL. Single component 226 Hz tympanometry was performed using a GSI 33 middle ear analyzer. Contralateral acoustic reflex thresholds were also be obtained and determined to be lower than 95 dB SPL for all subjects to ensure normal middle ear function.

Subjects were trained to induce NMEP by the researcher. The researcher demonstrated induction of NMEP by pinching her nose and swallowing. A comparison of the feeling in the ear to flying in a plane was made. The subject was asked to practice, and tympanometry was performed during the practices to confirm that the subject was capable of inducing NMEP (more negative than -50 daPa). If the subject was not able to produce or sustain NMEP, the subject was compensated for his or her time and dismissed from the study.

Personalized ear molds were made for each subject capable of producing NMEP using a Westone Silicast ear mold kit following the manufacturer's guidelines. An ER10 plastic probe tip was imbedded in the subject's ear mold to ensure a consistent DPOAE probe fit during manipulation of middle ear pressure. Ear molds were used because small shifts in probe position during DPOAE data collection make it difficult to separate changes in probe position from changes in middle ear impedance. Data collection took approximately 4 hours. Data was collected across 3 days so that the subject's Eustachian tube could function optimally. Subjects

were paid for their participation. The IRB committee at the City University of New York's Graduate Center approved the research protocol.

## **2.2. Tympanometry**

Tympanometry was performed with a 226-Hz probe tone. In tympanometry, variations in air pressure are created via an air pump. A microphone monitors the probe tone sound pressure level at the different pressures provided by the air pump. The peak of the tympanogram represents the point of greatest energy flow through the middle ear system. In the subjects with normal middle ear function, the Tympanic Peak Pressure (TPP), or ear canal pressure at which the peak of the tympanogram occurs, is around atmospheric pressure (0 daPa). Figure 1 (left) represents one subject's normal tympanogram. The peak of this normal tympanogram is at 5 daPa. If the TPP occurs at a negative pressure, it implies that the middle ear pressure is also negative. Figure 1 (right) represents one subject's negative TPP tympanogram, and the point of greatest energy flow occurs at -145 daPa.

TPPs more negative than -50 daPa were accepted as criteria for the NMEP condition based on Marshall et al. (1997) and Prieve, Calandruccio, Fitzgerald, Mazevski, & Georgantas (2008). For subjects capable of producing NMEP, tympanometry was performed at normal baseline pressure (8 estimates) and at subject-induced NMEP (8 estimates). Tympanometric measures at normal pressure and NMEP were interleaved, so NMEP might have varied.

## **2.3. Energy Reflectance**

Reflectance provides an alternative tool for evaluating middle ear function. Energy reflectance is tested with a broadband stimulus covering a wider range of frequencies than tympanometry. Reflectance is more sensitive to mass-loaded pathologies because it includes information about mid- and high-frequency sound transmission (e.g., Keefe & Simmons, 2003).

It is an estimate of the ratio of reflected power (sound energy not absorbed by the middle ear system) to incident sound energy presented to the ear. A value of 1.0 (100%) indicates that all the energy presented to the middle ear was reflected while a lower value indicates more energy was able to enter the middle ear (e.g., Feeney, Grant, & Marryott, 2003).

Reflectance data was collected using Mimosa Acoustics' Hear ID Middle-Ear Power Analyzer (MEPA3). Reflectance probe calibration was performed once daily before collecting measurements. Following manufacturer's guidelines, the probe tip was placed in a calibration cavity, and 4 measurements were made for each probe channel. The system then calculated the Thevenin pressure and impedance parameters based on the lengths of the 4 cavities. Both probe channels needed to fall within the accepted range of values at 90% or more of the test frequencies for a successful calibration. The ER-10C microphone and speaker probe assembly was then placed in the subject's ear. A chirp stimulus at 60 dB SPL was delivered into the ear to evaluate a broad range of frequencies (62 Hz to 13,000 Hz) at ambient pressure. An estimate of the proportion of the original stimuli reflected back into the ear canal was obtained. The subject was then asked to induce NMEP, and an additional set of measurements was collected. Reflectance measurements were interleaved at normal pressure (8 estimates with 8 trials per estimate) and NMEP (8 estimates with 8 trials per estimate).

## **2.4. DPOAE testing**

### **2.4.1. Stimuli**

Two primary tones were swept logarithmically (one second per octave) from  $f_1 = 410$ - $6560$  Hz and  $f_2 = 500$ - $8000$  Hz with a fixed primary ratio of  $f_1/f_2 = 1.22$ , producing  $2f_1-f_2$  from  $320$ - $5120$  Hz. Three equal-level primary level combinations were used (L65, L70, L75 dB SPL). High-level primaries were used because fewer sweeps were necessary to obtain good signal-to-

noise ratios (6 dB above the noise floor) at these levels. These signal-to-noise ratios were required to ensure that the DPOAEs were above the noise floor during NMEP, as NMEP increases the impedance of the middle ear system. Stimuli were calibrated using KEMAR (see Henin et al., 2011).

#### **2.4.2. Data collection**

Subjects were seated in a reclining chair in a double-walled sound-booth with their ear-molds snugly positioned in their ear. The presentation of stimuli and the recording of the ear canal signal were controlled by custom programs on a MAC computer (OS X) connected to a Mark of the Unicorn (MOTU) 828 Fire-wire Audio Interface. Computer generated stimuli were converted by the MOTU audio interface and impedance matched by Tucker-Davis Technologies (TDT) headphone buffers (HB6) connected through the booth wall to Etymotic Research (ER) ER2 insert headphones in the ER10 microphone assembly which was embedded in the subject's personalized ear mold and rested in the subject's test ear. The ER10 A microphone was used to convert the acoustic energy in the ear canal to an electrical signal which was sent to a battery-operated preamplifier and a battery-operated Stanford Research SR 560 low-noise amplifier / filter (L65 gain = 50; L70 gain = 10; L75 gain = 10; 300-10000 Hz band-pass filter). The MOTU 828 digitized the stimuli at a sampling rate of 44100 Hz before storing it on a hard disk for offline processing.

There were 8 trials at each equal-level primary combination (L65, L70, L75 dB SPL). Each trial contained 16 up-sweeps for each middle ear pressure as shown in Figure 2. Following collection at normal pressure, the subject induced NMEP using the Toynbee and signaled a successful maneuver by turning a flashlight on and off. After collection at NMEP, the subject swallowed and released the pressure change. An additional signal using the flashlight indicated

full pressure release and a final calibration was then collected. It was very important that the beginning and end calibrations were similar so that changes in DPOAE during NMEP were not confused with a change in probe position. If the final calibration was not consistent, the tester waited approximately 1 minute and performed the final calibration again. Usually, this resolved the problem as it gave the subject more time to fully release the change.

## **2.5. Analysis**

### **2.5.1. Tympanometry, reflectance, and change in DPOAE primaries**

Tympanometry, reflectance measures, and changes in primary  $f_1$  and  $f_2$  level and phase (Henin et al., 2011) were used to evaluate middle ear function at normal pressure and subject-induced NMEP.

Mean, range, and standard deviation of tympanometric peak pressure values were obtained for each subject. Estimates of the difference in reflectance (NMEP- Normal) as a function of frequency were obtained by subtracting the estimates of reflectance for each middle ear pressure at each frequency after a subtraction 1/3-octave band averaging was performed (320-5120 Hz) to reduce the number of data points.

Primary  $f_1$  and  $f_2$  level and phase were extracted from the ear canal recordings offline using a custom artifact rejection program in MATLAB (ver.2008b). A weighted-average procedure down weighted noisy segments of data. The artifact rejection program computes a power estimate of the noise around the frequency of interest, for example, the frequency of the generator component. Subsequently, a least squares fit (LSF) analysis procedure was used to estimate the level and phase of the DPOAE primaries and components.

Primary frequencies  $f_1$  and  $f_2$  should reflect similar processes and were visually similar. Visual comparison of  $f_1$  primary level at normal pressure and NMEP was performed on each trial

to confirm that a stable NMEP was induced. If a trial of 16 sweeps was not consistent with other NMEP trials (e.g., greater than 3 dB or one standard deviation from the mean), then the trial was removed from further analysis (no more than 4 trials were every removed from one condition). A more detailed evaluation of the effects of NMEP on composite DPOAE and the generator and reflection components was then performed.

### **2.5.2. Composite DPOAE and its components**

The same LSF procedure was used to evaluate composite DPOAE and its components. Overlapping windowed segments of data were analyzed (Long et al., 2008). The size of the analysis window determined the bandwidth of the analysis. A short (2756 point) filter was used to extract the level and phase of the composite DPOAE so both components fell within its window. A narrowband (11025 point) filter with a fixed latency was used to extract the generator component and an additional narrowband filter with a frequency-dependent latency was used to extract the reflection component (Long et al., 2009). DPOAE level (decibels (dB)) and phase (cycles) from valid trials were averaged.

Mean and standard deviation of DPOAE level (dB) at each frequency, primary level, and middle ear pressure tested for each subject were calculated using MATLAB (e.g., N1/L65/Normal pressure; N1/L65/NMEP). DPOAE level differences (dB) between NMEP and Normal were calculated and compared within subject (e.g., N1/L65/NMEP – N1/L65/Normal). DPOAE level differences (dB) from each primary were also compared within subject. A repeated measures ANOVA was performed on 3 factors, middle ear pressure (Normal vs. NMEP), primary level (L65, L70, L75 dB SPL), and frequency (500-4000 Hz). The same programs were used to evaluate level differences for the generator and reflection components.

Composite DPOAE and component phase was compared at Normal and NMEP at frequencies when the magnitude of the components was at least 6 dB above the noise floor (see Henin et al., 2011). Phase (cycles) was unwrapped when noise contamination induced shifts of more than one cycle. Phase difference was calculated (NMEP-Normal) for primaries  $f_1$  and  $f_2$ .

Finally, DPOAE spacing at NMEP and Normal were calculated and compared. The frequency (Hz) and level (dB) of the DPOAE fine-structure maxima were calculated using a custom written program in MATLAB (ver. 2008b) (see Henin et al., 2011). Changes were evaluated by calculating the difference in maxima at each middle ear pressure. Frequency changes were binned into frequency regions based on the Greenwood function ( $f_{BM}(x)=165.4 * (10^{(2.1*x/35)} - 1)$ ), which represents approximately 1mm spacing on the basilar membrane (Greenwood, 1961, a, b).

In summary, frequency (Hz) level (dB) changes in the DPOAE and its components at each middle ear pressure (Normal and NMEP) and primary level (L65, L70, L75 dB SPL) were analyzed using a LSF procedure. Phase (cycles) changes and differences in DPOAE fine-structure spacing at each middle ear pressure were also analyzed.

## **Chapter 3. Results**

Twenty-six subjects signed consent forms to participate in the study. However, only 8 subjects (30%) were able to successfully and consistently induce NMEP by performing the Toynbee maneuver. Data presented are from the 8 subjects that induced NMEP more negative than (-50 daPa), as measured by tympanometry.

### **3.1. Middle ear static pressure effects on middle ear function**

Middle ear pressure was estimated indirectly using 3 techniques; tympanic peak pressure (TPP), acoustic reflectance, and by comparison of changes between DPOAE primary tones in the ear canal at normal pressure and NMEP.

#### **3.1.1. Tympanometry**

Mean, range, and standard deviation TPPs for normal pressure and NMEP are presented in Table 1. Mean TPPs collected during NMEP ranged from -65 daPa (N11) to -324 daPa (N2) and averaged -185 daPa. The wide range of TPPs collected was useful because they reflect the variety of TPPs observed in clinical populations. A wide range of standard deviations per subject was observed during NMEP. Note that it was harder to obtain consistent TPP when larger NMEP was required.

#### **3.1.2. Reflectance measures**

Reflectance is tested with a broadband stimulus and covers a wider range of frequencies than tympanometry. It is an estimate of the ratio of reflected power (sound energy not absorbed by the middle ear system) to incident sound energy presented to the ear. Reflectance measures were collected to determine the impact of NMEP on estimates of cochlea reflection. These estimates are displayed in the upper (percent reflectance) and lower (phase) panels of Figures 3, 4, 5, and 6. By interleaving Normal and NMEP, an estimate of the amount and variability of

subject-specific changes in both percent reflectance and reflectance phase was obtained. During NMEP, all subjects demonstrated increased reflectance for a portion of the frequencies between 500 and 4000 Hz.

The frequency range most affected by NMEP was between 1000 and 1500 Hz with an average of 23% more energy reflected across subject (Figure 7). Individual and mean change in percent reflectance (NMEP-Normal) is displayed in Table 2. Subjects N8 (TPP -145 daPa) and N14 (TPP -129 daPa) showed the most reflectance at NMEP, almost 40% in this region. The frequency regions from 500 to 1000 Hz and 1500 to 2000 Hz also demonstrated large increases in reflectance at NMEP: 16.2% and 18.5%, respectively. The trend of increased reflectance continued to 4000 Hz. Beyond 4000 Hz there was a switch and subjects displayed increased reflectance during normal pressure compared to NMEP.

No consistent relationship between the amount of TPP induced and the percent of energy reflected was observed (Figure 1A of the Appendix). There was no correlation between degree of TPP negativity and change in percent reflectance at 500 to 1000 Hz (Pearson  $r = .090$ ;  $p = .415$ ), 1000 to 1500 Hz (Pearson  $r = .188$ ;  $p = 0.328$ ), 1500 to 2000 Hz (Pearson  $r = .349$ ;  $p = .198$ ), 2000 to 2500 Hz (Pearson  $r = -.203$ ;  $p = 0.314$ ), 2500 to 3000 Hz (Pearson  $r = -.363$ ;  $p = 0.188$ ), 3000 to 3500 Hz (Pearson  $r = -.373$ ;  $p = .181$ ), 3500 to 4000 Hz (Pearson  $r = .070$ ;  $p = 0.434$ ), 4000 to 4500 Hz (Pearson  $r = .408$ ;  $p = 0.157$ ), 4500 to 5000 Hz (Pearson  $r = .290$ ;  $p = .242$ ), 5000 to 5500 Hz (Pearson  $r = -.092$ ;  $p = 0.413$ ), and 5500 to 6000 Hz (Pearson  $r = -.129$ ;  $p = .380$ ).

For example, subject N11 (TPP -65 daPa) had an average increase in reflectance below 2000 Hz of 21% while N2 (TPP -324 daPa) reflected 22% of energy in this frequency region. Despite a difference of 259 daPa between N11 and N2, there was only a 1% difference in percent

reflectance. The frequencies affected by NMEP were also different for each subject. Subject N13 (TPP -262 daPa) reflected 22% of energy between 2000 and 4000 Hz while subject N1 (TPP -271 daPa) absorbed additional energy in this frequency region during NMEP. Both subjects had similar TPPs but displayed very different changes in reflectance. The effects of NMEP are subject-specific and TPP, which is measured at one low frequency, does not accurately represent middle ear reflectance/absorbance of sound across frequency.

Including reflectance phase enables a more complete picture of the system. Reflectance phase is shown in the lower portions of Figures 3 (subjects N1 and N2), 4 (subjects N4 and N6), 5 (subjects 8 and 11), and 6 (subjects 13 and 14). In subjects with small standard deviations, there was a clear difference in pattern of reflectance phase across frequency between middle ear pressures. The change in phase was consistent with the change in percent reflectance. Reflectance phase with NMEP lagged the phase at normal pressure until approximately 3000 Hz. Between 2500 Hz and 3500 Hz there was a switch and the phase at NMEP lead the phase when pressure was Normal. Also, the phase obtained with NMEP crossed zero at a lower frequency than the phase at normal pressure.

### **3.1.3. Change in DPOAE primary ( $f_1$ ) level**

With the system used, reflectance measures could not be collected simultaneously with DPOAE measures. To ensure that NMEP had been consistently induced during DPOAE data collection, changes in the level (dB SPL) and phase (cycles) of the  $f_1$  DPOAE primaries were evaluated. Similar to the estimates of reflectance, there was a subject-specific pattern of change in primary  $f_1$  level across frequency when NMEP was induced (shown in Figure 8). For every subject, at each primary (L65, L70, L75 dB SPL), an increased amount of energy remained in the ear canal and a decreased amount of energy entered the middle ear during NMEP because of the

increased impedance of the system below 1500 Hz. For example, between 1000 and 1500 Hz there was an average of 1.7 dB SPL more energy in the ear canal and less energy entered the middle ear during NMEP (shown in Table 3). From 1500 to 3500 Hz there was a switch and more energy entered the middle ear during NMEP. Beyond 3500 Hz less energy again entered the middle ear during NMEP and remained in the ear canal. Composite DPOAE and component level was reduced across frequency during NMEP despite increases and decreases in the amount of energy that entered the middle ear.

#### **3.1.4. Change in DPOAE primary phase**

Changes in the phase of the primary frequencies were also investigated and, as with the reflectance phase data, there were subject-specific patterns of change in phase (cycles) across frequency during NMEP. These patterns are displayed in Figure 9 for the L65 (dB SPL)  $f_1$  primary and in Figure 10 for the L65 (dB SPL) primary  $f_2$ . Within in each subject, the patterns for  $f_1$  and  $f_2$  were very similar because they were obtained at the same time.

For most subjects, the phase at normal pressure lead the phase at NMEP between 1000 and 2000 Hz. Between 2000 and 4000 Hz there was a change, and the phase at NMEP lead the phase at Normal pressure. The phase differences beyond 4000 Hz were less clear. The difference in phase between the two middle ear pressures (NMEP-Normal) for  $f_1$  and  $f_2$  are shown clearly in Figure 11. Although the pattern of phase differences was subject-specific, each subject had at least one large peak and this occurred around 3000 Hz. The frequency of the peak difference ranged from 2000 to 4000 Hz.

## 3.2. Middle ear static pressure effects on DPOAE

### 3.2.1. Composite DPOAE level and phase

Changes in composite DPOAE level and phase were analyzed to permit comparison of results with those from other researchers who did not separate the components. The composite DPOAE levels with and without NMEP (see Figure 12) were subject-specific and consistent across the three primaries tested. There were significant reductions in the composite DPOAE level as a function of frequency in all subjects. A three-way repeated measure ANOVA was conducted using IBM SPSS Statistics 20 for the effects of primary level (L65, L70, L75 dB SPL), middle ear pressure (Normal vs. NMEP), and frequency (500-4000 Hz). There was a significant main effect on composite DPOAE level as a function of the primaries ( $p < .00001$ ), middle ear pressure ( $p = .014$ ), and frequency ( $p < .00001$ ). There were no significant interactions between the factors (see appendix Table 1A).

Differences (NMEP-Normal) averaged across frequency for each subject and the average of all subjects are shown in Table 4 and Figure 13. Subject N2 (TPP -324 daPa) had the most average reduction of DPOAE level frequency followed by subject N1 (TPP -271 daPa) (Figure 12, left column). At L65 (dB SPL), N2's average composite DPOAE level at NMEP was -12.7 dB less frequency than at normal pressure. Subject N1's composite DPOAE level was -10.7 dB lower during NMEP. Subject N14 (TPP -129 daPa) demonstrated the least amount of change, -1 dB, followed by subject N11 (TPP -65 daPa) (Figure 12, right column).

RMS levels of eight frequencies bins between 1060 and 3537 Hz for primary L65, L70, and L75 dB SPL are shown in Table 5 and in Figure 14 for primary L65 dB SPL. The frequency range in each bin was determined according to the Greenwood function ( $f_{BM}(x)=165.4 * (10^{(2.1*x/35)} -1)$ ), which represents approximately 1mm spacing on the basilar membrane

(Greenwood, 1961, a, b). Frequencies below 1000 Hz were not included because of significant noise contamination in this region. The frequency region between 2644 and 3060 Hz had the greatest amount of change in level during NMEP for all subjects, with mean decrease in level of -5.8, -5.6, and -5.1 dB for the primaries L65, L70, and L75 dB SPL, respectively. The six frequency bins below this region also had decreases in DPOAE level at NMEP across the primaries tested. The smallest change in composite DPOAE level during NMEP was observed above 3060 Hz.

Composite DPOAE phases were similar across subject and are displayed in Figure 15. For both middle ear pressures there was a gradual slope, which indicates that the generator component was the dominant component in the composite DPOAE.

### **3.2.2 Change in DPOAE fine-structure width and depth**

Changes in DPOAE fine-structure width and depth were estimated as it was hypothesized that an increase in middle ear impedance caused by NMEP would lead to wider DPOAE spacing and deeper DPOAE maxima and minima. Estimates of DPOAE maxima and minima for every subject and primary level tested at normal pressure and NMEP were determined using a custom Matlab program (ver. 2008b), which picks DPOAE maxima and minima. The points were visually inspected to confirm that analysis included corresponding frequency maxima and minima. Visual inspection also allowed the researcher to confirm that estimates due to noise were not included in analysis.

The fine-structure maxima were calculated for every subject, middle ear pressure, and primary level and are shown in Table 2A of the Appendix. Larger frequency ranges between points of maxima for NMEP compared to Normal would be indicative of wider DPOAE spacing during NMEP. Visual inspection of Figure 12 and graphs representing all subjects similar to

those in Figure 12, did not confirm any systematic change in spacing between points of maxima across level, frequency, or middle ear pressure. Figure 16 depicts the fine structure maxima (Hz) for subject N1 and normal pressure and NMEP. A three way repeated measures ANOVA on primary level (L65, L70, L75 dB SPL), frequency band (8 bands from 1060-3537 Hz), and middle ear pressure (Normal vs. NMEP) was performed (IBM SPSS Statistics 20) for every subject and is shown in the Table 4A of the Appendix. Statistics confirmed the visual interpretation. There was no significant difference in fine-structure width between Normal and NMEP (see Table 4A of the Appendix).

The fine-structure depths for every subject, middle ear pressure, and primary level were also obtained. This was done by subtracting points of fine-structure minima from corresponding points of maxima for every subject and primary level. The fine-structure depths for each subject were then binned by frequency according to the Greenwood function (Greenwood, 1961, a, b). Individual mean and standard deviation DPOAE peak depths (dB) for each frequency band are in the Table 3A of the Appendix. Fine structure peak depth (dB) for N1 at normal pressure and NMEP is shown in Figure 17. A three way repeated measures ANOVA on primary level (L65, L70, L75 dB SPL), frequency band (8 bands from 1060-3537 Hz), and middle ear pressure (Normal vs. NMEP) was performed for each subject. Statistics confirmed the visual conclusions. There was no significant affect of primaries, frequency band, or middle ear pressure on the DPOAE peak depth shown in the (see Table 4A of the Appendix).

### **3.2.3. Effects on generator component level and phase**

Composite DPOAE analysis indicated that the generator component was dominant. Separation of the components of the composite DPOAE will therefore aid interpretation of NMEP effects. Removal of the reflection component from the composite DPOAE provided

analysis of the generator component alone (see Figure 18). This permitted better estimates of the effects of NMEP, as the variance of the generator component data was reduced. Generator component level changes during NMEP followed the same pattern as composite DPOAE changes. The decrease in generator component level and pattern of change during NMEP was subject-specific, consistent, and greater than the composite DPOAE. A three-way repeated measure ANOVA was conducted for the effects of primary level (L65, L70, L75 dB SPL), middle ear pressure (Normal vs. NMEP), and frequency (500-4000 Hz). There was a significant main effect on generator component level of the primaries ( $p < .00001$ ), middle ear pressure ( $p = .010$ ), frequency ( $p < .00001$ ), and there were no significant interactions between factors (see Table 1A of the appendix).

Average generator component level changes (NMEP-Normal) for every subject are in Figure 19. At L65 dB SPL the generator component level at NMEP was an average of -12 dB lower in level for both N2 (TPP -324 daPa) and N1 (TPP -271 daPa) (Table 6). Subject N14 (TPP -129 daPa) demonstrated an average change in level of only -1 dB at L65 dB SPL.

RMS levels in eight frequencies bins between 1060 and 3537 Hz are shown in Table 7. Generator component levels were reduced at all frequencies in some subjects, but only in specific frequency regions in other subjects, Table 7 presents changes in RMS level (NMEP-Normal) within indicated frequency regions for the primaries L65, L70, L75 dB SPL. Subject N2 (TPP -324 daPa) again demonstrated the most change in generator component level across the widest range of frequencies followed by N1 (TPP -271 daPa). The frequency region most affected by NMEP was between 2644 and 3060 Hz with a decrease in generator component level during NMEP of -6.0, -5.7, and -5.1 dB for primaries L65, L70, and L75 dB SPL, respectively.

Every frequency bin demonstrated a decrease in generator component level during NMEP at all primaries of at least -3.5 dB (Table 7).

Generator component phases were similar to composite DPOAE phase and are displayed in Figure 20. Phase at Normal and NMEP were essentially flat across frequency after an initial slope between 500 and 2000 Hz. The slope was greater with NMEP.

#### **3.2.4. Effects on reflection component level and phase**

The generator component was at least 10 dB higher in level than the reflection component and relatively smooth across frequency when compared to the reflection component, which had a pattern of maxima and minima (e.g. Kalluri and Shera, 2001). Reflection component levels were closer to the noise floor than the composite DPOAE and generator component levels for both Normal and NMEP (see Figure 21). Changes between middle ear pressures across the primaries L65, L70, L75 dB SPL and frequency were still detected. A three-way repeated measure ANOVA was conducted for the effects of primary level (L65, L70, L75 dB SPL), middle ear pressure (Normal vs. NMEP), and frequency (500-4000 Hz). For the reflection component level, there was a significant main effect of the primaries ( $p = .015$ ) and frequency ( $p < .00001$ ), and middle ear pressure ( $p = .024$ ). Again, there was no significant interaction between factors (see Table 1A of the Appendix).

The estimated change was less than for the composite DPOAE and generator component. Individual mean data is shown in Table 8. There was a -7 dB decrease in level during NMEP at L65 (dB SPL) for both N1 and N2. Subjects N4 (-123 daPa), N6 (-157 daPa), and N14 (-129 daPa) demonstrated negligible change in level across frequency at L65 dB SPL. Also, the frequency region most affected was different for each primary, which was not the case for the composite DPOAE or the generator component (see Table 9). The pattern of reflection

component phase change across frequency was much steeper than the phases of the composite DPOAE and generator component and appeared to be similar for both middle ear pressures (see Figure 22).

Separation of the components improved analysis. When the reflection component was removed, the variability in generator component level was reduced. Larger, clearer, and more consistent changes were observed in the generator component alone than in the composite DPOAE or reflection component.

## **Chapter 4. Discussion**

### **4.1. Introduction**

Eight subjects were able to consistently induce NMEP by performing the Toynbee maneuver. During NMEP the average tympanometric peak pressure (TPP), was -185 daPa and ranged from -65 daPa to -324 daPa (see Table 1). The wide range of TPP obtained allowed detailed analysis of NMEP affects on composite and component DPOAE. Reflectance estimates added information about NMEP in the ear canal over a wider range of frequencies than 226 Hz tympanometry. There was an increase in percent reflectance for all subjects for a portion of the frequencies tested from 500 to 4000 Hz (see Figures 3, 4, 5, & 6; Table 3). The frequency region from 1000 to 1500 Hz was most affected by NMEP with almost 23% more energy reflected (see Table 3). There was no direct relationship between degree of TPP and percent reflectance (see Figure 1A of the Appendix).

Changes in primary level (dB SPL) and phase (cycles) in the ear canal served as a tool to ensure that consistent NMEP was maintained throughout data collection. There was a subject-specific pattern of change in level and phase of the primaries used to evoke the DPOAE, which was similar to the estimates of reflection. The pattern of change in level included both increases and decreases in the amount of energy that entered the middle ear during NMEP. The greatest reduction in energy occurred for frequencies below 1500 Hz (see Figure 8, Table 2). Despite increases and decreases in the amount of energy that entered the middle ear during NMEP, composite and component DPOAE levels were reduced at all frequencies.

There were significant reductions in composite DPOAE level for all subjects. This includes subject N11 (TPP -65 daPa), this TPP is considered clinically normal in some settings

(e.g. Jerger, 1970). The frequency region between 2644 and 3060 Hz was most affected by NMEP, but the six frequency bins below this region were also affected (see Figure 14, Table 5). The phase of the composite DPOAE was dominated by the generator component. To prevent contamination of estimates of the impact of NMEP by the DPOAE fine structure, the components were separated to improve analysis. When the components were separated, there were larger and more consistent effects of NMEP on the generator component.

#### **4.2. Estimates of middle ear static pressure**

Tympanometric measures were collected because they are common in adult clinical practice (for review see Margolis & Hunter, 1999) and were useful when training subjects to induce NMEP. A TPP of -100 daPa is considered indicative of normal middle ear function most clinical settings (e.g., Jerger, 1970); however, Marshall et al. (1997) found that this degree of negativity or less (i.e., -31 to -65 daPa) significantly reduced TEOAE levels. Therefore, a range of TPP from -50 to -400 daPa was used as criteria for NMEP and abnormal middle ear function. The range of TPPs collected in this study was between -65 daPa and -324 daPa. There were significant changes in composite DPOAE and component level for every subject, regardless of TPP, indicating that TPPs, -50 daPa and more negative, will decrease DPOAE level and may affect DPOAE test results.

Although there was significant correlation between the amount of negative TPP induced and reduction in generator component at primary L65 dB SPL (Pearson  $r = .847$ ;  $p = 0.004$ ), L70 (Pearson  $r = .782$ ;  $p = 0.01$ ), and L75 (Pearson  $r = .875$ ;  $p = 0.022$ ) the relationship was not simple (Figure 2A of the Appendix). For example, subject N14 (TPP -129 daPa) demonstrated the least amount of change in level across frequency though this subject did not have the least negative TPP. Additionally, five subjects with TPPs that ranged from -65 daPa to

-157 daPa displayed approximately the same degree of reduction in composite and component amplitude across frequency. The two subjects that did induce the most negative TPP (N1, -271 daPa and N2, -324 daPa) did show the greatest decrease in DPOAE level during NMEP. Other investigators have found a significant relationship between the amount of negative TPP induced and degree of EOAE level reduction (Osterhammel et al., 1993; Plinkert et al., 1994; Robinson & Haughton, 1991; Sun & Shaver, 2009). Sun and Shaver (2009) collected DPOAEs from 16 adults with TPP from -40 to -420 daPa. DPOAE level was reduced for all subjects, and the reductions were greater for subjects with TPPs more negative than -160 daPa. We agree with Sun and Shaver (2009) that DPOAE level decreased most when TPP were greater than -160 daPa. Prieve et al. (2008), found no correlation between negativity as measured by TPP and amount of change in TEOAE level when collecting TEOAE from a large group of infants at regular intervals between 4 weeks of life and 2 years; eleven infants displayed NMEP at some point during the two-year period. Prieve et al. (2008) attributed the lack of correlation to the subject-specific nature of the ear canal, middle ear, and cochlea or in the case of infants, TEOAE level maturation.

Single-frequency tympanometry provides information at only one low frequency, 226 Hz, and requires the ear canal to be pressurized, which makes a poor test for newborns (Keefe et al., 2003). Wideband reflectance measures are a reliable supplement or possible alternative to tympanometry for both adults and children (e.g., Allen, Jeng, & Levitt, 2005; Feeney et al., 2003; Hunter et al., 2008; Shahnaz, Miranda, & Polka, 2008) because energy reflectance can be tested over a broad range of frequencies (62 Hz – 13,000 Hz) and does not require the ear canal to be pressurized. In healthy ears without middle ear pathology, more incident power is absorbed between 1000 and 5000 Hz than at frequencies below 1000 Hz or above 5000 Hz (e.g.,

Allen et al., 2005; Feeney et al., 2003; Hunter et al., 2008; Shahnaz et al., 2008). In subjects with middle ear pathology, the amount of energy reflected and absorbed varies across frequency and depends on the nature of the pathology (Feeney et al., 2003).

Every subject had clear changes reflectance measures during NMEP with the lowest frequencies tested being most affected (see Figure 7). This is because NMEP pulls the TM inward and increases the stiffness reactance of the middle ear system. Stiffness reactance is inversely proportional to frequency. Therefore, NMEP was expected to decrease the transmission of low-frequency sounds more than high-frequency sounds, which it did. The frequency region most affected by NMEP was 1000 to 1500 Hz. The frequency bands directly below 1000 Hz and above 1500 Hz also demonstrated increased reflectance (see Table 3). Beyond 2000 Hz there was less energy reflected off the TM during NMEP, and from 4000 to 6000 Hz more energy entered the middle ear. Voss, Merchant, and Horton (2011) manipulated the middle ear air space of cadaveric ear with positive and negative static pressure and found significant increased reflectance for frequencies below 2000 Hz, but not above. Feeney et al., (2003) collected reflectance measures from three subjects with Otitis media with effusion and one subject with NMEP. The subjects showed increased percent reflectance estimates for frequencies below 4000 Hz, though the subject with NMEP was only affected up to 1000 Hz, while the effects for the subjects with Otitis media with effusion were much greater (Feeney et al., 2003).

No consistent relationship between the amount of TPP induced and percent reflectance was found (Figure 1A of the Appendix). For example, subject N8 (TPP -145 daPa) had the greatest increase in energy reflectance during NMEP, almost 40% between 1000 and 1500 Hz, and subject N13 (TPP -262 daPa) was affected across the greatest range of frequencies (1000-

4000 Hz). Shaver and Sun (2011) measured the effects subject-induced (Toynbee maneuver) NMEP on reflectance measures in 48 subjects. There was a weak correlation between reflectance measures and negative TPP values (Shaver & Sun, 2011). As TPP is measured at 226 Hz, the lack of correlation may be because a great deal of energy is already reflected in the low frequency region around 226 Hz or because energy reflectance is specific to the complexity of an individual's ear canal and middle ear.

TPP and reflectance estimates could not be used to confirm NMEP during DPOAE testing. Instead, the level and phase of the primary tone  $f_1$  used to evoke the DPOAE at normal pressure and at NMEP were compared to confirm on a trial-by-trial basis that NMEP had been induced. This method has been used to separate middle ear muscle contraction from efferent activation caused by contralateral acoustic stimulation (Deeter, Abel, Calandruccio, L, & Dhar, 2009; Henin et al., 2011). Contraction of the middle ear muscle reflex has some similarity to NMEP because it also affects the input signal reaching the cochlea as it changes the impedance of the middle ear system.

There were clear, consistent, and subject-specific changes in both primary level and phase of the evoking stimuli between normal pressure and NMEP (see Figure 8). The change in primary level was systematic, yet different in magnitude as a function of frequency for each subject. The subject specific change in pattern was used to indicate that NMEP had been induced during DPOAE data collection.

Changes in the level of  $f_1$  indicated there was an increase in the amount of energy that was reflected at low frequencies with less energy entering the middle ear. This was followed by a decrease in energy in the ear canal in the frequency region around 2000 Hz (indicating more energy entered the middle ear). There was an increase in energy in the ear canal frequencies

beyond 3000 Hz again indicating less energy entered the middle ear during NMEP. The increases and decreases in primary energy across frequency are consistent with an increase in middle ear resonance during NMEP pressure. This is similar to the pattern seen when the middle ear muscle contracted (Henin et al., 2011). Feeney and Keefe (1999) modeled the shift in middle ear resonance during contraction of the middle ear muscle with reflectance measures. The frequency changes observed in their reflectance data are similar to the changes observed in our reflectance measures and changes in  $f_1$ .

#### **4.3. Composite and component DPOAE and middle ear static pressure**

Despite the pattern of both increase and decrease in primary  $f_1$  level as a function of frequency during NMEP, the composite DPOAE level was consistently lower across frequency. For example, between 1500 and 3000 Hz there was an overall increase in energy (approximately 2 dB) that entered the middle ear during NMEP, yet there were decreases in composite and component DPOAE level of approximately -4 to -5 dB for these frequencies. Overall, there was a decrease in composite DPOAE level during NMEP, which continued beyond 3000 Hz and was not solely concentrated in the low frequencies.

The affects of NMEP on composite DPOAE level steadily increased from 1000 Hz to 3000 Hz, which is contrary to the results of Sun (2011) which found greatest affects below 1000 Hz and affects that lessened thereafter, to a minima at 2000 Hz. Sun and Shaver (2009) also observed a minimal reduction in composite DPOAE level during NMEP at approximately 2000 Hz and suggested that this was the resonant frequency of the middle ear system during NMEP. In this data, the frequency band containing 2000 Hz, and the bands below and above, had large decreases in composite DPOAE level, and results are not in agreement with Sun (2011) and Sun and Shaver (2009). Sun and Shaver (2009) used a program that calibrated the primaries at the

entrance of the ear canal, which are modified by standing waves in the ear canal at quarter-wave frequencies (e.g., Siegel, 1994). This leads to an incorrect adjustment of primary levels. We estimate the levels at the eardrum (see Long et al., 2008), but not the level reaching the cochlea. In this data, composite and component DPOAEs were reduced in level despite increased primary levels entering the cochlea. The decreases in composite and component level, even when the primaries were expected to increase in level in the cochlea, may be a result of changes in reverse transmission (transfer of distortion products from the cochlea to the ear canal) but we cannot be sure as our technique does not allow for the separation of forward and reverse transmission.

To better separate the effects of changes in forward and reverse transmission on DPOAE levels during NMEP, we need to correct for changes in the forward transmission of the primaries. Forward pressure level (FPL) is an alternative to the sound pressure level calibration, and is performed in-situ. This method separates the incident (FPL) and reflected energy components. This is done by prior determination of the Thevenin-equivalent source impedance and pressure of the transducer (for review see Lewis, McCreery, Neely, & Stelmachowicz, 2009). Calibration with dB forward pressure level reduces OAE variability when compared to dB sound pressure level calibration (Scheperle et al., 2008).

Without the ability to separate the effects of NMEP on forward and reverse transmission, changes in the DPOAE fine structure were analyzed. It was hypothesized that when the energy generated in the cochlea has difficulty exiting the middle ear it would be re-reflected back to its characteristic frequency place. Reflections and re-reflections of energy were expected to create a standing wave pattern in the cochlea and modify the structure of the DPOAE (Long et al., 2011). For subjects with large reflection components at higher primary levels, the re-reflections were expected to enhance some frequencies and cancel others producing a wider pattern of DPOAE

maxima and minima with negative group delay. Research by Long et al., 2011 on six subjects with impacted cerumen and one subject with two separate incidents of NMEP caused by separate long distant flights demonstrated this. No significant changes in DPOAE depth or width were observed in this data and the lack of large reflection components in the subjects in this experiment may partially explain why.

Another possible explanation may lay in the variation of middle ear pressure over time due to the interleaved episodes of normal pressure and NMEP. Each change in pressure in the middle ear could be expected to evoke a pressure change in the cochlea, which in turn would modify basilar membrane properties. The change in middle ear pressure may bias the position of the basilar membrane, pushing it up or down, and this may change the properties of cochlear nonlinearity and thus OAE generation and reflection. When the middle ear pressure is stable, for example NMEP due to a long-distance flight, this is less likely to be a problem because the heliocyte will allow the pressure difference between the oval and round windows to stabilize so that the basilar membrane will remain in its normal position. The equalization process takes time therefore it may not have been present using this interleaved technique. In addition to FPL, performing this study again on persons with stable NMEP may help with the problems that come from using the Toynbee maneuver.

Although FPL was not used, an advantage of this study was the analysis of phase data, which is also often overlooked. The composite DPOAE phase indicated that the generator component was dominant at the stimulus levels used. However, there was still fine structure, which indicates that the ear canal levels were also modified by reflection components. Any change in the relative levels of the two components could contaminate estimates of the effects of NMEP.

When the components were separated by additional analysis, another advantage of this study, there was less variability due to component mixing resulting in a smoother function with fewer levels peaks and valleys. Changes in generator component level were clearer and less variable but followed the same pattern as the changes in composite DPOAE. For example, when the primary level was 65 dB SPL, the mean decrease in generator component level was approximately -5.2 dB at 2000 Hz, -5.2 dB at 2500 Hz and -4.6 dB at 3200 Hz, which was more than that observed for the composite DPOAE. As expected, the proximity of the reflection component lead to less consistent and more difficult to interpret change due to NMEP.

There was a statistically significant change in composite and component DPOAE levels in each subject. This indicates that even small amounts of NMEP had an affect on DPOAEs. The level difference between middle ear pressures and the frequencies affected by NMEP were subject-specific and would likely affect the results obtained using the any clinical device.

Most clinical devices (particularly those in neonatal screening programs) measure discrete, widely spaced frequency DPOAEs. Currently, infants with NMEP may “refer” at one or more of the lower frequencies tested, possibly measured in DPOAE fine-structure minima. The “refer” would not be a result of cochlear pathology, but due to increased impedance in the middle ear system and the nature of DPOAE generation. For example, across subject at primary level L65 dB SPL, there was an approximate decrease in composite DPOAE level of -5 dB at 2000 Hz, -5 dB at 2500 Hz and -2.5 dB at 3200 Hz. These average level reductions were measured across subjects’ DPOAE fine structure, which includes level maxima and minima. If the discrete measurement were collected at a minimum with the Natus Echo-Screen, then NMEP would much more likely result in a “refer” than if the DPOAE was collected at a maximum. However, the clinician currently has no way of discerning this information. The generator

component may therefore provide more reliable results than the composite DPOAE or the reflection component. Evaluation of the generator component would improve test validity, as the clinician can be sure the data collected is not measured in a DPOAE minimum.

### **4.3. Conclusion**

The Toynbee maneuver provides consistent and replicable changes in middle ear pressure and is a useful technique for studying the affects of NMEP on DPOAEs. Reflectance measures indicate that during NMEP more energy is reflected off the tympanic membrane and less energy enters the cochlea. This is especially true for frequencies below 2000 Hz. Reflectance measures could not be collected simultaneously with DPOAE measures. However, changes in DPOAE primary level and phase were a reliable indicator of subject-induced NMEP.

There was a correlation between amount of TPP induced and change in generator component level. During NMEP there is a consistent reduction in composite DPOAE and component level across frequency and TPP (-65 daPa to -324 daPa) despite primary  $f_1$  level data, which indicated that some frequency regions had an increase in the amount of energy that entered the middle ear during NMEP. Composite DPOAE and component changes are subject-specific and consistent across the levels tested (L65, L70, L75 dB SPL). The effects were clearer and easier to interpret for the generator component because there is less variance than in the composite DPOAE.

## 5. Tables

*Table 1.* Tympanic peak pressure (TPP) mean, standard deviation, and range for each subject capable of consistently producing NMEP from least amount of NMEP (N11) to greatest (N2).

Subject N4 was the only male.

Subject	Normal			NMEP		
	TPP (daPa)	SD	Range (daPa)	TPP (daPa)	SD	Range (daPa)
N11 (F)	4	4	-5 to 10	-65	12	-80 to -50
N4 (M)	4	3	0 to 10	-123	18	-150 to -90
N14 (F)	1	13	-15 to 20	-129	30	-185 to -90
N8 (F)	6	7	0 to 20	-145	46	-201 to -55
N6 (F)	-1	6	-10 to 5	-157	13	-180 to -140
N13 (F)	-8	7	-5 to 0	-262	39	-345 to -230
N1 (F)	1	9	-20 to 5	-271	30	-305 to -230
N2 (F)	0	9	-20 to 25	-324	95	-385 to -120
Avg.	1	7	-20 to 25	-185	35	-385 to -50

Table 2. Individual mean and standard deviation change in percent reflectance (NMEP – Normal) are displayed. Means across subject were also calculated. Data is grouped in 500 Hz frequency bins. Across subject there was a large increase in energy reflected from the middle ear below 2000 Hz, and the frequency range between 1000 and 1500 Hz was most affected with almost 23% more energy reflected during NMEP. Subject N8 reflected almost 40% more energy during NMEP between 1000 and 1500 Hz.

	.5-1 kHz		1-1.5 kHz		1.5-2 kHz		2-2.5 kHz		2.5-3 kHz		3-3.5 kHz		3.5-4 kHz		4-4.5 kHz		4.5-5 kHz		5-5.5 kHz		5.5-6 kHz	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
N1	10.5	1.7	11.2	1.1	13.4	0.8	2.0	6.4	-6.9	2.8	-0.6	3.3	-7.3	1.0	-4.1	0.4	-3.9	0.8	-2.9	0.8	-6.1	0.9
N2	20.1	4.4	27.2	1.6	18.5	4.2	14.4	1.2	8.4	3.6	3.6	0.7	-0.7	3.9	-19.9	5.7	-25.6	1.3	-21.7	0.9	-21.8	0.8
N4	-1.7	1.9	3.8	1.4	12.4	3.8	17.6	0.7	13.5	1.5	8.9	0.8	6.2	0.9	-2.8	5.0	-28.1	9.8	-54.4	5.1	-57.2	2.3
N6	20.1	6.1	26.7	7.8	12.2	3.7	1.8	1.4	4.2	1.0	3.0	1.9	-5.1	1.0	-0.9	2.3	1.4	0.7	-1.7	1.4	-10.0	2.3
N8	32.8	4.0	39.3	4.8	27.5	14.2	-3.8	2.2	1.6	1.5	13.6	4.0	18.0	2.8	5.2	2.9	-4.8	3.7	-19.3	5.1	-35.1	4.2
N11	14.8	2.7	22.6	1.3	24.0	1.8	8.0	7.0	-10.1	4.4	-8.0	5.5	-0.6	1.1	-6.8	1.8	-10.6	0.6	-8.8	1.6	-4.7	0.6
N13	7.5	2.6	14.0	0.9	16.8	0.7	18.4	1.1	23.5	1.3	24.5	1.1	18.8	2.5	5.9	6.6	-19.0	5.3	-21.1	2.4	-17.4	2.0
N14	25.4	7.5	38.2	4.3	22.8	5.9	11.6	5.4	2.6	3.7	-7.7	2.2	5.1	3.7	7.7	2.2	-3.6	3.8	-12.2	1.2	-11.2	1.5
Avg.	16.2	11	22.9	13	18.5	5.79	8.7	8.1	4.6	11	4.7	11	4.3	9.8	-2.0	8.9	-11.8	11	-17.8	17	-20.4	18

Table 3. Individual mean and standard deviation change in decibels (NMEP – Normal) for primary ( $f_1$ ) at L65 dB SPL are displayed. Means across subject were also calculated. Positive values indicate an increase amount of energy that remained in the ear canal and did not get transferred to the cochlea during NMEP. Frequencies below 1500 Hz and above 3500 Hz were most affected by NMEP.

	.5-1 kHz		1-1.5 kHz		1.5-2 kHz		2-2.5 kHz		2.5-3 kHz		3-3.5 kHz		3.5-4 kHz		4-4.5 kHz		4.5-5 kHz		5-5.5 kHz		5.5-6 kHz	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
N1	1.5	0.3	0.4	0.2	-1.7	1.9	-5.4	3.9	1.3	0.3	1.8	0.1	1.4	0.2	0.8	0.1	0.7	0.1	0.1	0.2	-0.6	0.3
N2	0.4	0.6	2.6	0.4	1.0	0.8	-0.9	0.2	-2.7	0.9	-7.4	1.5	-1.0	1.4	1.2	0.5	2.6	1.3	4.7	0.3	3.5	0.5
N4	0.2	0.4	2.1	1.7	-2.7	0.5	-2.2	0.5	-0.1	1.0	2.3	0.5	1.9	0.3	0.7	0.7	-0.4	0.8	-0.9	0.2	-0.2	0.1
N6	2.3	1.3	0.3	1.4	-1.9	0.7	-2.3	0.3	-2.3	1.0	1.3	0.3	1.6	0.4	0.7	0.0	0.7	0.0	0.8	0.1	0.7	0.1
N8	2.4	1.1	3.4	0.8	-2.8	1.4	-3.9	0.3	-3.7	0.0	-2.7	1.1	0.9	1.1	3.8	0.4	4.2	0.4	2.0	1.0	-0.8	0.6
N11	-0.1	0.2	1.2	0.3	-0.9	1.2	-3.0	0.9	1.9	1.0	0.6	0.4	0.4	0.0	0.2	0.1	0.1	0.1	-0.1	0.0	-0.2	0.0
N13	1.1	0.8	1.6	0.7	-0.1	0.3	-2.0	1.0	-6.3	1.9	1.3	1.3	2.4	0.1	2.2	0.0	1.8	0.4	0.7	0.2	0.4	0.0
N14	1.1	1.0	2.4	1.6	-2.6	0.6	-4.5	1.2	0.7	1.4	2.3	0.7	1.3	0.3	1.8	0.5	0.4	1.3	-2.1	0.2	0.0	0.7
Avg.	1.1	0.9	1.7	1.1	-1.5	1.4	-3.0	1.5	-1.4	2.8	-0.1	3.4	1.1	1.1	1.4	1.1	1.3	1.5	0.7	2	0.4	1.4

*Table 4.* Composite DPOAE level change in decibel (NMEP – Normal) averaged across frequency for every subject. The three primary levels (L65, L70, L75 dB SPL) tested are represented. Negative values indicated a decrease in composite DPOAE level during NMEP. Across frequency, subject N2 was most affected by NMEP. For subject N2 at L65 dB SPL, the composite DPOAE level was -12.7 dB less across frequency during NMEP compared to Normal.

	TPP (daPa)	L65 (dB SPL)		L70 (dB SPL)		L75 (dB SPL)	
		Mean (dB)	SD	Mean (dB)	SD	Mean (dB)	SD
<b>N11</b>	-65	-2.7	3.2	-2.8	3.5	-1.9	2.2
<b>N4</b>	-123	-1.8	2.6	-1.2	2.5	-1.4	2
<b>N14</b>	-129	-0.1	2.8	-1.0	2.3	-0.1	1.5
<b>N8</b>	-145	-1.7	5.1	-5.0	6.5	-2.7	4
<b>N6</b>	-157	-1.4	2.1	-1.6	1.8	-1.4	1.6
<b>N13</b>	-262	-4.2	2.3	-5.0	3.7	-5.3	4.6
<b>N1</b>	-271	-10.7	2.7	-7.7	2.7	-5.4	2
<b>N2</b>	-324	-12.7	4.4	-8.3	3	-11.5	3.9
<b>Average</b>		-4.4	4.68	-4.1	2.88	-3.7	3.67

Table 5. Individual mean and standard deviation composite DPOAE level changes (dB) (NMEP – Normal) for L65, L70, and L75 dB SPL are displayed. Means across subject were also calculated. Data is binned by frequency according to the Greenwood function. Across subject and primary, the frequency region between 2644 and 3060 Hz was most affected by NMEP with an average decrease in DPOAE level of -5 dB.

L65 (dB SPL)								
	1060-1242 (Hz)	1243-1450 (Hz)	1451-1690 (Hz)	1691-1965 (Hz)	1966-2281 (Hz)	2282-2643 (Hz)	2644-3060 (Hz)	3061-3537 (Hz)
N1	-13.79	-10.50	-9.03	-11.42	-12.30	-12.92	-10.51	-5.21
N2	-5.06	-12.30	-13.90	-13.25	-10.34	-11.14	-15.24	-20.64
N4	-3.15	0.47	1.54	0.12	-3.78	-5.18	-4.62	-0.06
N6	-1.35	0.34	1.12	0.64	-1.24	-2.34	-3.52	-4.77
N8	-3.40	-4.22	-3.23	-0.50	1.33	-3.15	-9.11	-8.40
N11	-0.99	-1.92	-6.30	-7.88	-4.20	-1.98	0.10	1.74
N13	-6.81	-3.83	-3.49	-3.67	-5.06	-6.88	-4.05	0.37
N14	-1.13	0.24	-2.55	-4.13	-4.94	0.54	0.85	3.57
Mean	-4.46	-3.96	-4.48	-5.01	-5.07	-5.38	-5.76	-2.08
SD	4.28	4.96	5.16	5.32	4.44	4.68	5.49	8.68
L70 (dB SPL)								
	1060-1242 (Hz)	1243-1450 (Hz)	1451-1690 (Hz)	1691-1965 (Hz)	1966-2281 (Hz)	2282-2643 (Hz)	2644-3060 (Hz)	3061-3537 (Hz)
N1	-9.22	-6.91	-7.96	-9.32	-10.03	-9.34	-7.11	-1.74
N2	-4.95	-11.22	-9.85	-5.68	-6.04	-7.44	-13.51	-7.86
N4	0.68	1.94	0.24	0.05	-3.04	-5.56	-3.47	-0.70
N6	-2.87	-0.48	0.26	0.55	-0.86	-1.65	-3.62	-4.13
N8	-11.11	-8.95	-7.13	-5.63	-3.55	-5.07	-8.51	10.04
N11	-0.21	-1.92	-5.90	-8.95	-4.88	-2.48	0.34	1.46
N13	-7.77	-4.71	-5.88	-5.32	-5.80	-7.85	-6.81	3.74
N14	-3.22	2.03	2.82	-1.60	-3.52	-2.02	-1.69	-0.49
Mean	-4.83	-3.78	-4.17	-4.49	-4.72	-5.18	-5.55	0.04
SD	4.24	4.98	4.62	3.80	2.72	2.91	4.37	5.34
L75 (dB SPL)								
	1060-1242 (Hz)	1243-1450 (Hz)	1451-1690 (Hz)	1691-1965 (Hz)	1966-2281 (Hz)	2282-2643 (Hz)	2644-3060 (Hz)	3061-3537 (Hz)
N1	-7.19	-5.00	-5.28	-6.05	-7.13	-6.05	-5.38	-0.90
N2	-8.60	-16.17	-13.21	-7.81	-7.40	-8.81	-17.25	-12.85
N4	1.65	0.52	-1.57	0.14	-2.66	-4.23	-2.66	-2.04
N6	-2.53	-0.30	0.46	0.31	-1.04	-1.03	-3.42	-3.43
N8	-5.04	-4.13	-5.39	-4.39	-1.80	-2.77	-4.54	6.65
N11	-1.40	-0.18	-3.74	-5.09	-4.38	-1.57	-0.18	1.02
N13	-11.12	-6.55	-6.02	-5.38	-5.51	-6.24	-6.93	5.25
N14	-0.12	2.16	0.82	-1.52	-2.55	0.01	-0.37	0.89
Mean	-4.29	-3.71	-4.24	-3.72	-4.06	-3.83	-5.09	-0.68
SD	4.44	5.88	4.49	3.01	2.43	3.04	5.43	6.00

Table 6. Individual mean and standard deviation generator component level difference in dB (NMEP – Normal) averaged across frequency at the three primaries tested. Subject N2 was most affected by NMEP and subject N14 was least affected. The amount of change was consistent across primary levels tested. More change was observed in the generator component compared to the composite DPOAE.

	TPP (daPa)	L65 (dB SPL)		L70 (dB SPL)		L75 (dB SPL)	
		Mean (dB)	SD	Mean (dB)	SD	Mean (dB)	SD
<b>N11</b>	-65	-2.1	3.5	-2.2	3.8	-1.7	2.4
<b>N4</b>	-123	-2.2	2	-1.6	2.3	-1.4	2
<b>N14</b>	-129	-1.1	2.6	-1.1	2.2	-0.3	1.3
<b>N8</b>	-145	-4	3.5	-7.6	2.6	-4.5	1.5
<b>N6</b>	-157	-1.5	2.1	-1.8	1.8	-1.5	1.6
<b>N13</b>	-262	-4.3	2	-6	1.4	-6.5	1.7
<b>N1</b>	-271	-11.8	2.9	-7.8	2.5	-5.5	1.9
<b>N2</b>	-324	-12.1	3.7	-8.8	3.2	-12.2	4.6
<b>Average</b>		-4.9	4.5	-4.6	3.24	-4.2	3.92

Table 7. Individual mean and standard deviation generator component level changes (dB) (NMEP – Normal) for L65, L70, and L75 dB SPL are displayed. Means across subject were also calculated. Data is binned by frequency according to the Greenwood function. Across subject and primary level, the frequency region between 2644 and 3060 Hz was most affected by NMEP.

<b>L65 (dB SPL)</b>								
	<b>1060-1242 (Hz)</b>	<b>1243-1450 (Hz)</b>	<b>1451-1690 (Hz)</b>	<b>1691-1965 (Hz)</b>	<b>1966-2281 (Hz)</b>	<b>2282-2643 (Hz)</b>	<b>2644-3060 (Hz)</b>	<b>3061-3537 (Hz)</b>
<b>N1</b>	-15.24	-12.56	-12.81	-12.34	-12.39	-13.05	-10.70	-5.18
<b>N2</b>	-6.37	-14.78	-16.21	-11.27	-8.47	-9.58	-16.33	-13.87
<b>N4</b>	-2.62	-0.31	-0.60	-0.51	-3.53	-5.10	-4.70	-0.54
<b>N6</b>	-1.66	0.17	0.87	0.54	-1.13	-2.17	-3.55	-5.26
<b>N8</b>	-3.87	-4.24	-3.17	-0.58	0.86	-3.08	-9.20	-8.42
<b>N11</b>	-1.67	2.44	-5.96	-7.26	-4.46	-2.03	-0.03	1.87
<b>N13</b>	-6.58	-4.26	-3.49	-3.81	-4.77	-6.77	-4.28	-0.29
<b>N14</b>	-1.83	0.09	-2.54	-4.12	-4.60	-0.07	0.87	3.08
<b>Mean</b>	-4.98	-4.18	-5.49	-4.92	-4.81	-5.23	-5.99	-3.58
<b>SD</b>	4.61	6.31	5.99	4.94	4.11	4.36	5.76	5.72
<b>L70 (dB SPL)</b>								
	<b>1060-1242 (Hz)</b>	<b>1243-1450 (Hz)</b>	<b>1451-1690 (Hz)</b>	<b>1691-1965 (Hz)</b>	<b>1966-2281 (Hz)</b>	<b>2282-2643 (Hz)</b>	<b>2644-3060 (Hz)</b>	<b>3061-3537 (Hz)</b>
<b>N1</b>	-9.09	-7.67	-8.43	-9.21	-9.79	-9.02	-7.08	-1.94
<b>N2</b>	-5.82	-13.18	-10.73	-5.73	-6.11	-7.57	-13.17	-7.85
<b>N4</b>	-0.18	1.50	-0.68	-0.32	-3.04	-5.37	-3.91	-0.89
<b>N6</b>	-3.18	-0.72	0.08	0.44	-0.82	-1.65	-3.71	-4.52
<b>N8</b>	-11.42	-8.89	-7.34	-5.73	-3.94	-4.88	-8.54	-10.17
<b>N11</b>	-0.97	2.69	-5.90	-7.71	-5.15	-2.56	0.08	1.76
<b>N13</b>	-7.94	-5.31	-5.83	-5.48	-5.58	-7.58	-6.90	-3.57
<b>N14</b>	-3.68	1.55	2.42	-1.46	-3.33	-2.12	-1.63	-0.58
<b>Mean</b>	-5.28	-3.75	-4.55	-4.40	-4.72	-5.09	-5.61	-3.47
<b>SD</b>	3.98	5.85	4.62	3.53	2.65	2.80	4.21	3.96
<b>L75 (dB SPL)</b>								
	<b>1060-1242 (Hz)</b>	<b>1243-1450 (Hz)</b>	<b>1451-1690 (Hz)</b>	<b>1691-1965 (Hz)</b>	<b>1966-2281 (Hz)</b>	<b>2282-2643 (Hz)</b>	<b>2644-3060 (Hz)</b>	<b>3061-3537 (Hz)</b>
<b>N1</b>	-7.31	-5.74	-5.26	-6.27	-6.71	-5.79	-5.47	-1.08
<b>N2</b>	-9.19	-20.25	-13.80	-8.21	-7.55	-8.94	-17.01	-12.64
<b>N4</b>	2.06	-0.36	-1.11	0.09	-2.64	-4.19	-2.81	-1.96
<b>N6</b>	-2.82	-0.54	0.30	0.19	-0.94	-1.18	-3.47	-3.60
<b>N8</b>	-5.55	-4.32	-5.26	-4.46	-2.27	-2.57	-4.56	-6.94
<b>N11</b>	-1.41	1.55	-3.29	-4.84	-4.34	-1.81	-0.17	1.05
<b>N13</b>	-10.21	-6.84	-6.04	-5.44	-5.10	-5.96	-6.86	-5.20
<b>N14</b>	-0.60	1.61	0.88	-1.54	-2.25	-0.88	-0.43	0.69
<b>Mean</b>	-4.38	-4.36	-4.20	-3.81	-3.98	-3.91	-5.10	-3.71
<b>SD</b>	4.38	7.19	4.69	3.07	2.35	2.83	5.34	4.54

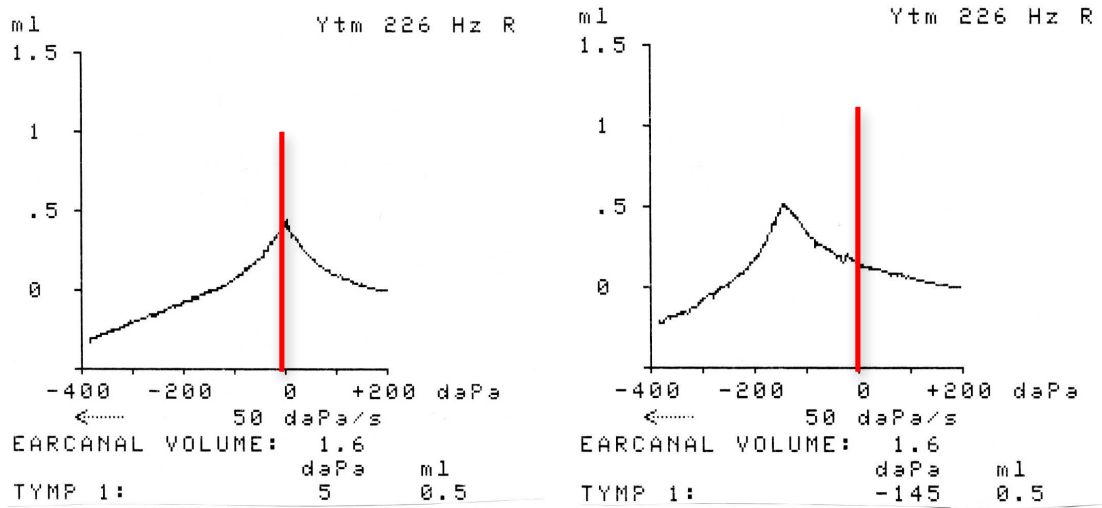
Table 8. Individual mean and standard deviation reflection component level difference in dB (NMEP – Normal) averaged across frequency at the three primary levels tested. Less of a change is observed for the reflection component between middle ear pressures because the level of the reflection component was closer to the noise floor for both middle ear pressures. Subject N2 demonstrated the most change while subject N6 was least affected by NMEP.

	TPP (daPa)	L65 (dB SPL)		L70 (dB SPL)		L75 (dB SPL)	
		Mean (dB)	SD	Mean (dB)	SD	Mean (dB)	SD
<b>N11</b>	-65	-1.9	3	-2	3	-1.7	2.3
<b>N4</b>	-123	-0.3	1.5	0.5	2.1	-0.6	1.9
<b>N14</b>	-129	-0.6	3.4	-0.8	2.9	0.3	1.8
<b>N8</b>	-145	-1.3	1.9	-3.4	2.3	3.5	2.5
<b>N6</b>	-157	0.5	2.2	0.6	1.3	-0.1	1.5
<b>N13</b>	-262	-4.5	2.1	-5.8	1.7	-5.9	2.7
<b>N1</b>	-271	-6.8	2	-4.4	3	-3.8	2.9
<b>N2</b>	-324	-6.7	3.3	-5.7	3.2	-8	4.2
<b>Average</b>		-2.7	2.9	-2.6	2.6	-2.0	3.7

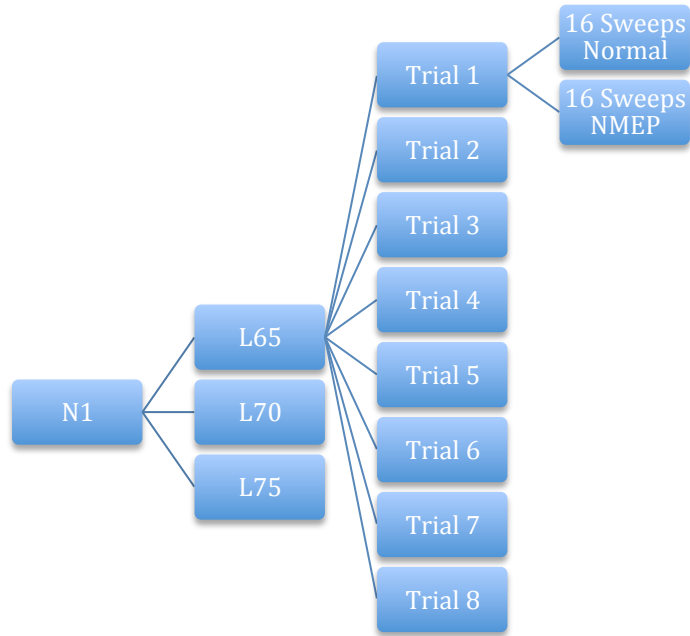
Table 9. Individual mean and standard deviation reflection component level changes (dB) (NMEP – Normal) for L65, L70, and L75 dB SPL are displayed. Means across subject were also calculated. Across subject and primary level every frequency region had a decrease in reflection component level during NMEP, but the decrease was less than when compared to the composite DPOAE and generator component.

<b>L65 (dB SPL)</b>								
	<b>1060-1242 (Hz)</b>	<b>1243-1450 (Hz)</b>	<b>1451-1690 (Hz)</b>	<b>1691-1965 (Hz)</b>	<b>1966-2281 (Hz)</b>	<b>2282-2643 (Hz)</b>	<b>2644-3060 (Hz)</b>	<b>3061-3537 (Hz)</b>
<b>N1</b>	-10.52	-6.67	-6.98	-6.15	-7.82	-6.20	-6.93	-3.22
<b>N2</b>	-9.10	-8.40	-12.08	-8.98	-3.40	-4.30	-3.70	-3.86
<b>N4</b>	-2.11	-0.11	1.08	1.18	-0.34	-1.31	-2.25	1.36
<b>N6</b>	0.51	2.30	3.44	1.25	1.33	-0.71	0.28	-4.05
<b>N8</b>	-0.40	-0.47	-2.65	-1.06	-0.73	0.66	0.01	-5.38
<b>N11</b>	1.40	0.70	-6.31	-4.69	-5.28	-0.41	-0.01	-0.51
<b>N13</b>	-7.16	-3.32	-4.23	-4.25	-5.83	-6.88	-0.60	-3.55
<b>N14</b>	4.47	-0.65	-1.84	-4.68	-4.71	0.46	-1.09	3.60
<b>Mean</b>	-2.86	-2.08	-3.70	-3.42	-3.35	-2.34	-1.79	-1.95
<b>SD</b>	5.43	3.74	4.88	3.60	3.15	3.01	2.47	3.12
<b>L70 (dB SPL)</b>								
	<b>1060-1242 (Hz)</b>	<b>1243-1450 (Hz)</b>	<b>1451-1690 (Hz)</b>	<b>1691-1965 (Hz)</b>	<b>1966-2281 (Hz)</b>	<b>2282-2643 (Hz)</b>	<b>2644-3060 (Hz)</b>	<b>3061-3537 (Hz)</b>
<b>N1</b>	-8.17	-2.86	-1.35	-3.20	-6.05	-7.59	-6.24	0.03
<b>N2</b>	-8.16	-8.44	-7.72	-1.69	-0.13	-4.57	-8.28	-6.46
<b>N4</b>	0.55	4.05	1.28	0.76	2.31	-1.37	-1.03	-2.39
<b>N6</b>	-0.83	1.70	3.20	0.06	-0.58	-0.23	0.82	0.42
<b>N8</b>	-6.94	-4.86	-3.12	-1.73	-0.97	-0.44	-3.83	-5.42
<b>N11</b>	1.95	1.48	-4.49	-4.58	-6.39	-2.07	-0.58	-1.24
<b>N13</b>	-6.93	-2.37	-4.55	-4.87	-6.18	-7.09	-7.61	-6.53
<b>N14</b>	2.38	-1.50	3.90	-1.64	-2.71	-5.11	-0.07	-1.71
<b>Mean</b>	-3.27	-1.60	-1.61	-2.11	-2.59	-3.56	-3.35	-2.91
<b>SD</b>	4.70	4.00	4.11	2.01	3.30	2.93	3.63	2.83
<b>L75 (dB SPL)</b>								
	<b>1060-1242 (Hz)</b>	<b>1243-1450 (Hz)</b>	<b>1451-1690 (Hz)</b>	<b>1691-1965 (Hz)</b>	<b>1966-2281 (Hz)</b>	<b>2282-2643 (Hz)</b>	<b>2644-3060 (Hz)</b>	<b>3061-3537 (Hz)</b>
<b>N1</b>	-6.91	-0.68	-1.99	-3.56	-5.67	-6.88	-5.29	0.86
<b>N2</b>	-12.97	-12.19	-13.00	-4.98	-2.53	-4.36	-6.69	-7.48
<b>N4</b>	1.14	2.24	0.51	0.21	-1.61	-2.44	-3.04	-1.61
<b>N6</b>	-1.19	0.27	2.75	0.53	-2.26	0.14	-1.00	-0.28
<b>N8</b>	-0.53	-5.39	-6.42	-4.96	0.02	-2.19	-5.87	-3.04
<b>N11</b>	1.12	-2.87	0.07	-5.04	-4.48	-2.31	-0.42	0.04
<b>N13</b>	-10.04	-2.54	-6.64	-4.85	-4.75	-6.88	-9.02	-2.87
<b>N14</b>	2.70	0.82	0.30	-3.00	-1.60	1.34	1.26	0.83
<b>Mean</b>	-3.34	-2.54	-3.05	-3.21	-2.86	-2.95	-3.76	-1.69
<b>SD</b>	5.85	4.58	5.23	2.33	1.93	2.98	3.54	2.79

## 6. Figures



*Figure 1.* Example of a normal tympanogram (left) and subject-induced NMEP tympanogram (right) collected from one subject. The peak of the tympanogram represents the point of greatest energy flow. The red line marks atmospheric pressure. The normal tympanic peak pressure (left) is 5 daPa. The tympanic peak pressure for the subject-induced NMEP is negative (-145 daPa).



*Figure 2.* A schematic diagram representing DPOAE collection for 1 subject at 3 levels with 8 trials per level and 16 sweeps per middle ear pressure.

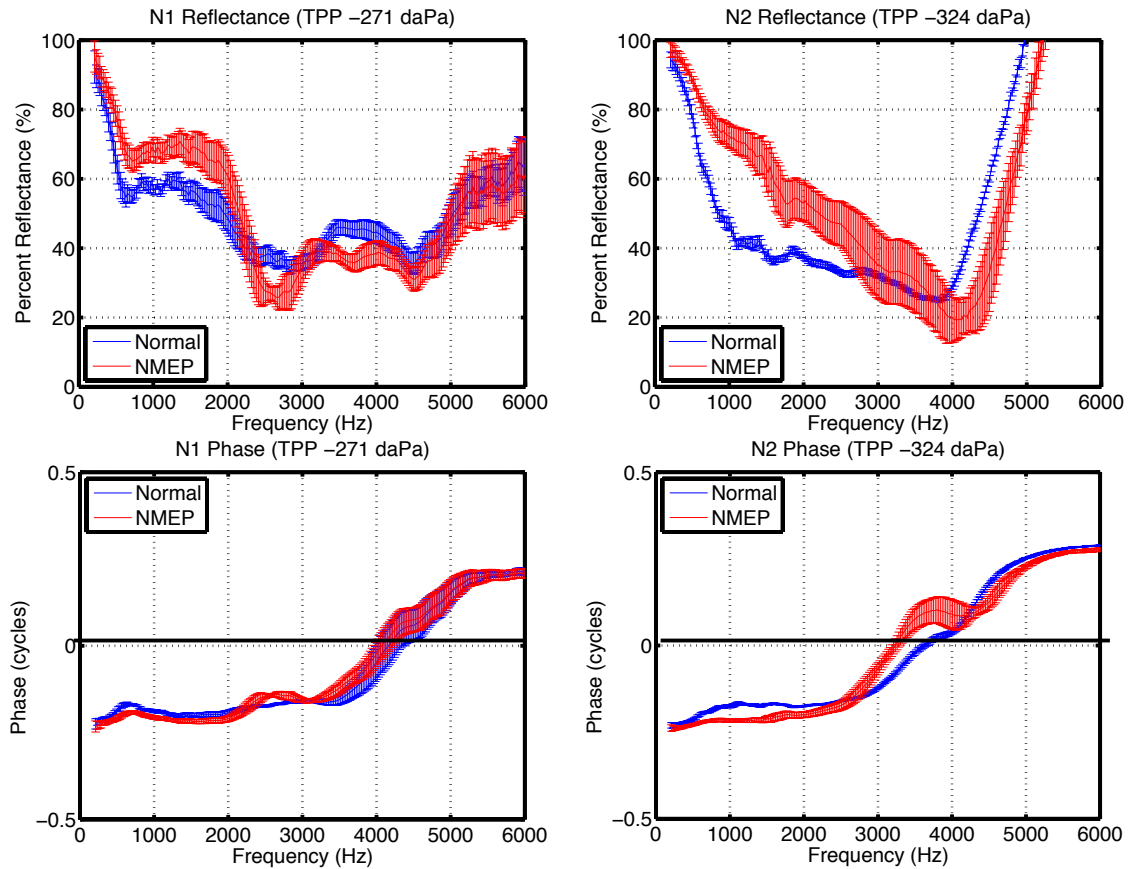


Figure 3. Mean and standard deviation of the 16 interleaved percent reflectance measures (top panels) and corresponding phase measures (bottom panels) for subjects N1 and N2 at normal pressure (blue) and NMEP (red). More energy is reflected from the TM below approximately 2000 Hz during NMEP. The phase crosses zero at a lower frequency for the NMEP.

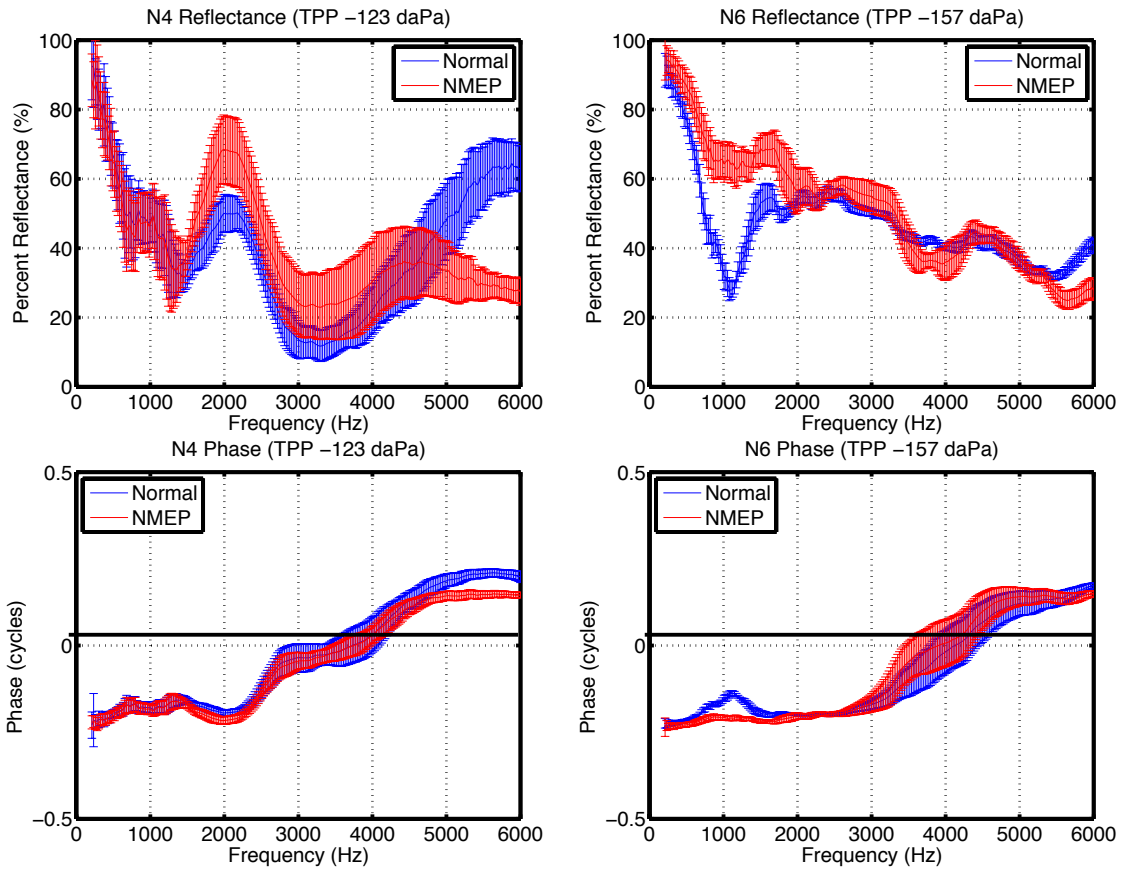


Figure 4. Mean and standard deviation of the 16 interleaved percent reflectance measures (top panels) and corresponding phase measures (bottom panels) for subjects N4 and N6 at normal pressure (blue) and NMEP (red).

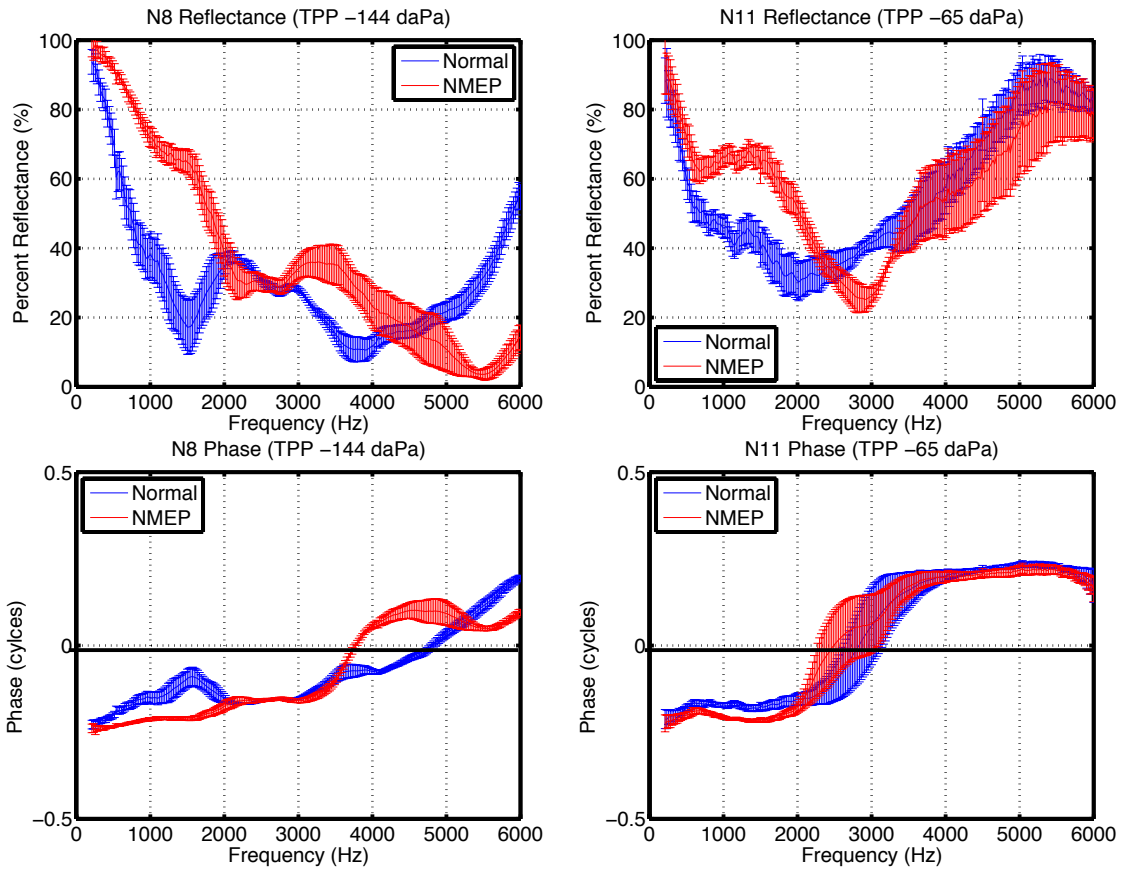


Figure 5. Mean and standard deviation of the 16 interleaved percent reflectance measures (top panels) and corresponding phase measures (bottom panels) for subjects N8 and N11 at normal pressure (blue) and NMEP (red).

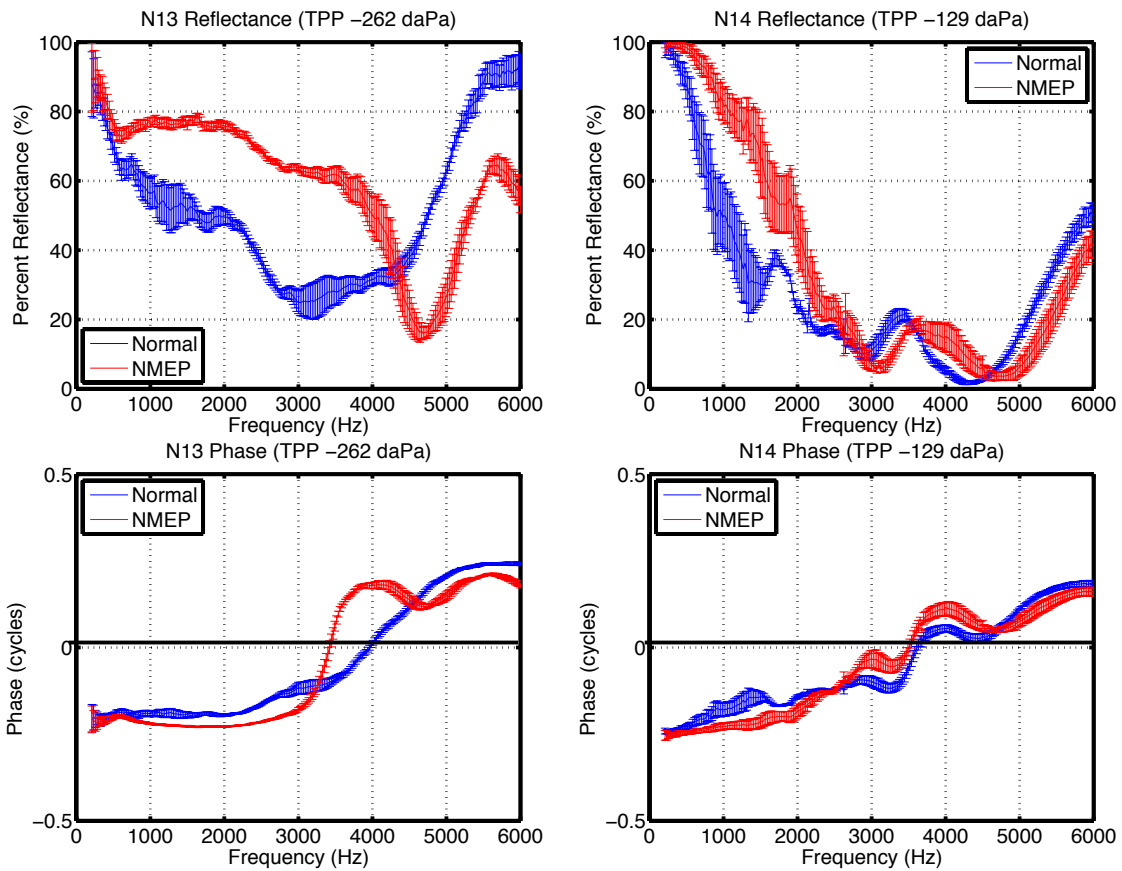
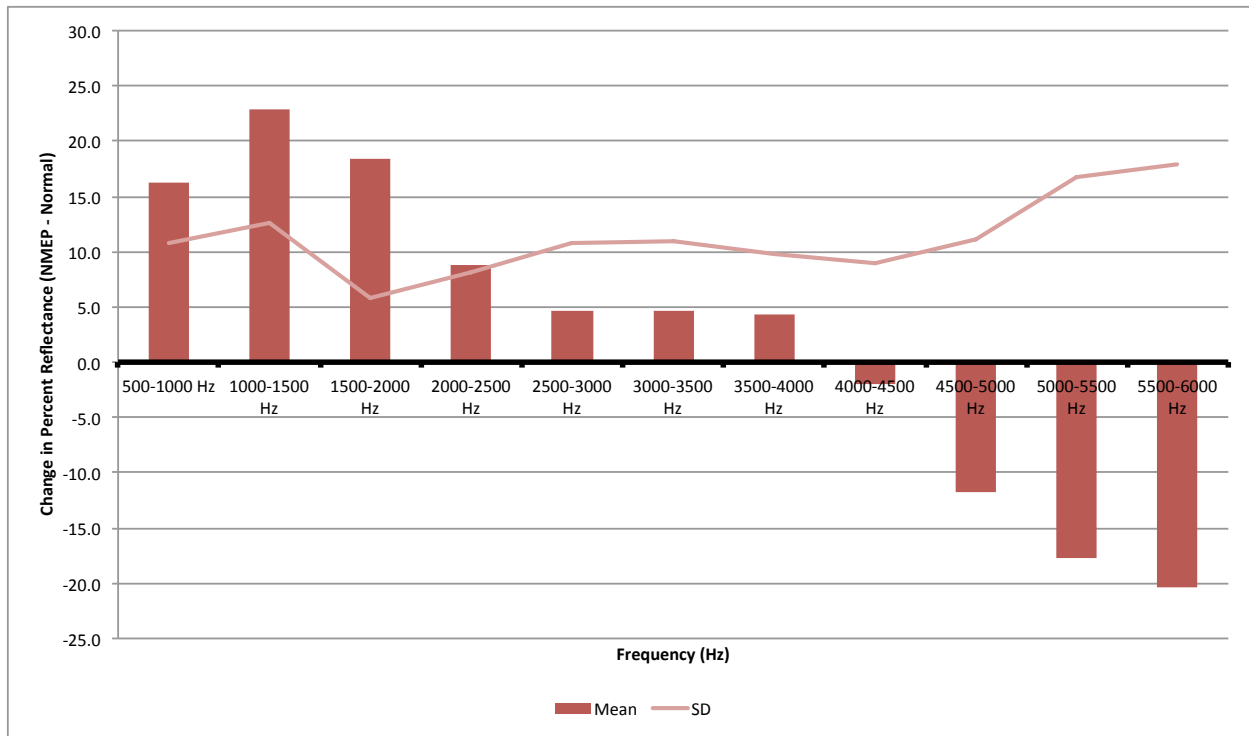
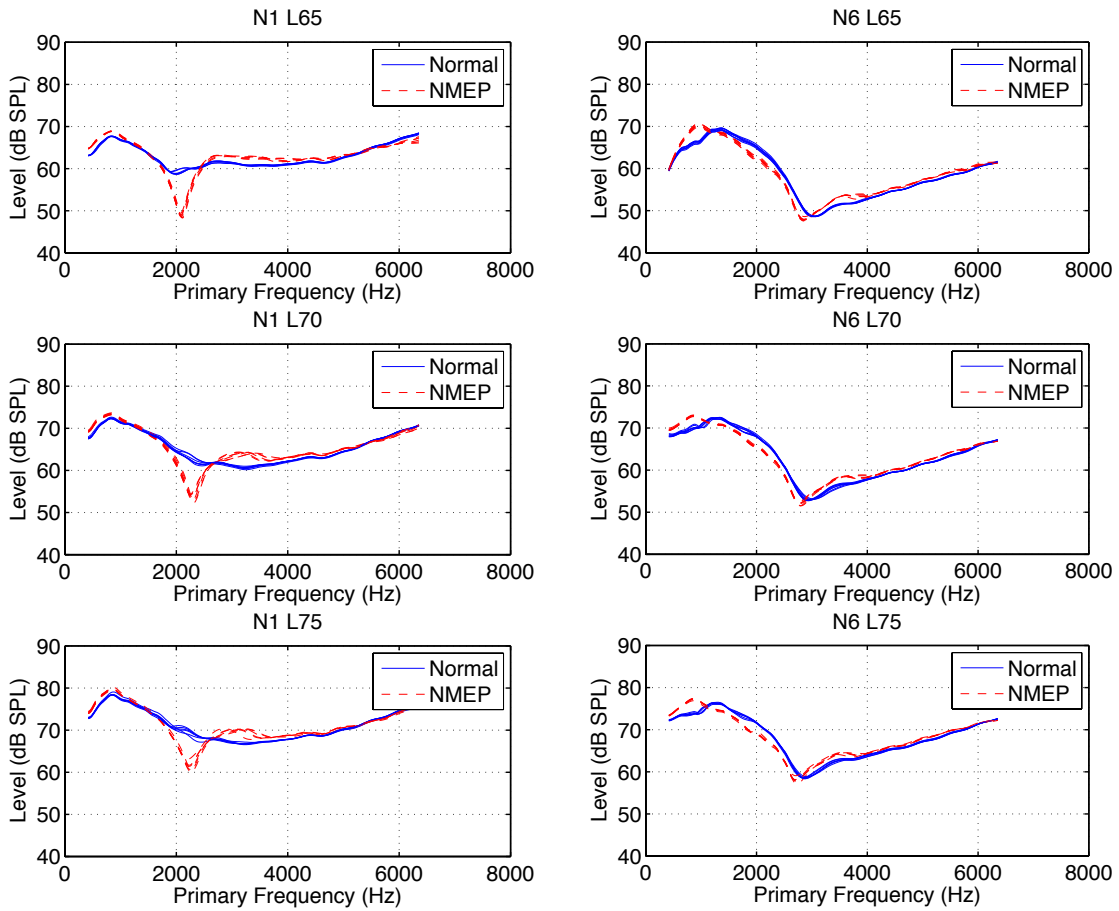


Figure 6. Mean and standard deviation of the 16 interleaved percent reflectance measures (top panels) and corresponding phase measures (bottom panels) for subjects N13 and N14 at normal pressure (blue) and NMEP (red).



*Figure 7.* Mean and standard deviation change in percent reflectance (NMEP – Normal) averaged across subject. There was a large increase in energy reflected during NMEP below approximately 2000 Hz. The frequency range 1000 to 1500 Hz was most affected with almost 23% more energy reflected during NMEP. Beyond 4500 Hz there was a switch and more energy entered the middle ear during NMEP.



*Figure 8.*  $f_1$  level (dB SPL) at the three primaries tested (L65, L70, L75 dB SPL) for subjects N1 (TPP -271 daPa), and N6 (TPP -157 daPa). Normal trials are represented with blue lines and NMEP trials with the red dashed lines. All subjects demonstrated a change in the amount of energy that entered the middle ear during NMEP, and each subjects' pattern of change is consistent across the primaries tested. For frequencies below 1500 Hz, less energy entered the middle ear and more energy remained in the ear canal during NMEP. This is consistent with the theory that increased stiffness leads to less low-frequency sound transmission.

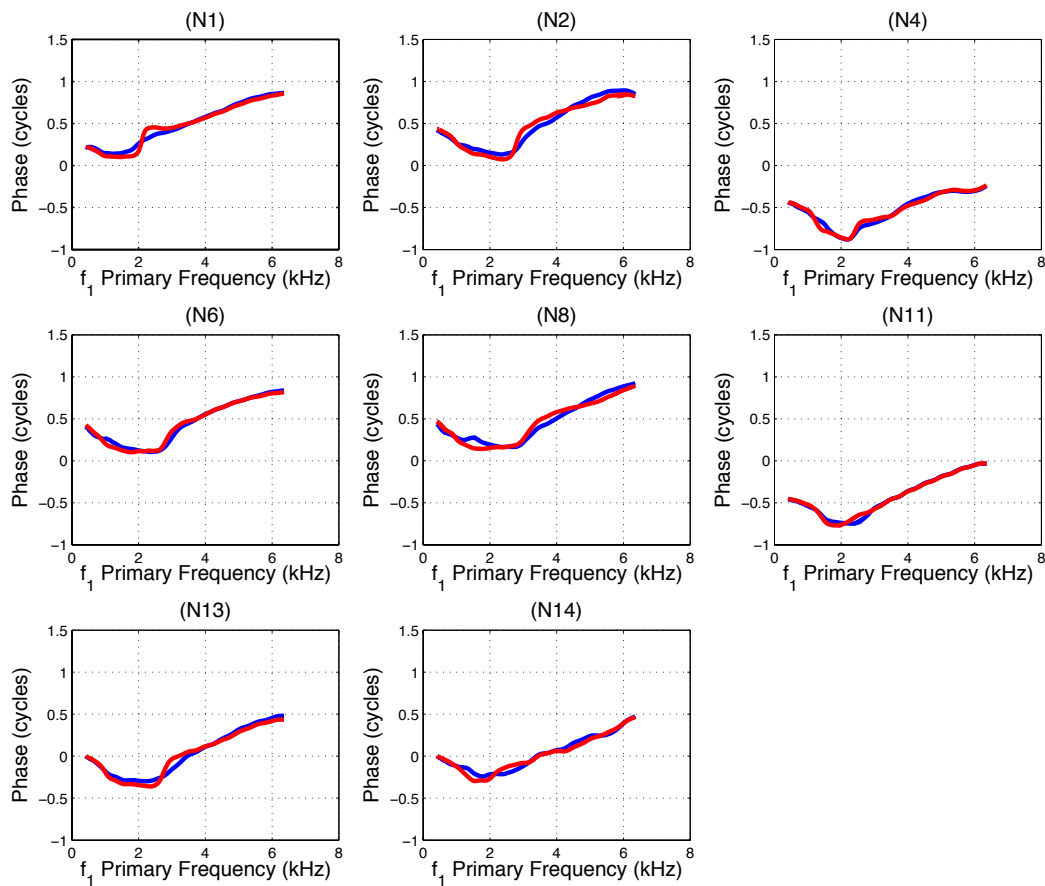


Figure 9. Primary  $f_1$  phase (cycles) at primary level L65 (dB SPL) for normal pressure (blue) and NMEP (red) across frequency for every subject are displayed above. Clear phase differences can be observed between Normal and NMEP. The change in primary  $f_1$  level in Figure 8 is reflected in the figure above for N1, and N6. For example, in subject N1 at NMEP there is a phase lag followed by a phase lead at approximately 2000 Hz.

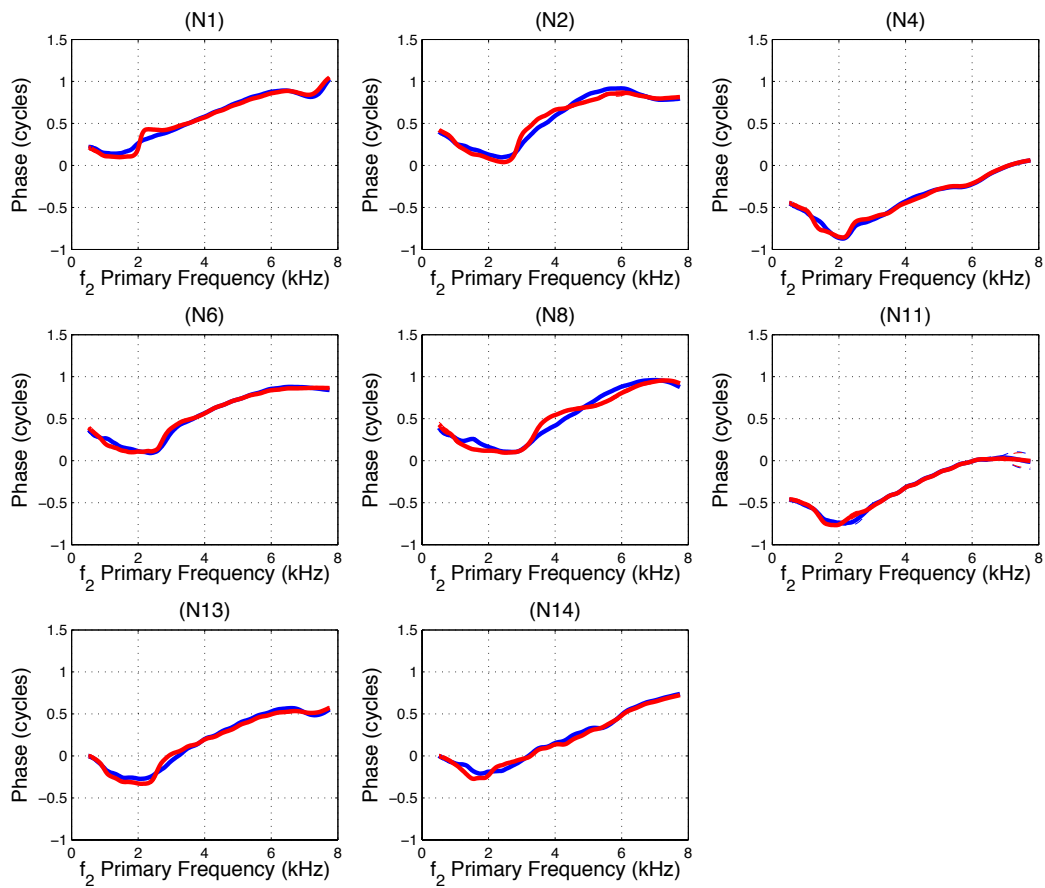


Figure 10. Primary  $f_2$  phase (cycles) at primary level L65 (dB SPL) for normal pressure (blue) and NMEP (red) across frequency for every subject are displayed above. Both  $f_1$  and  $f_2$  exhibited similar patterns of phase change across frequency and are not fully independent

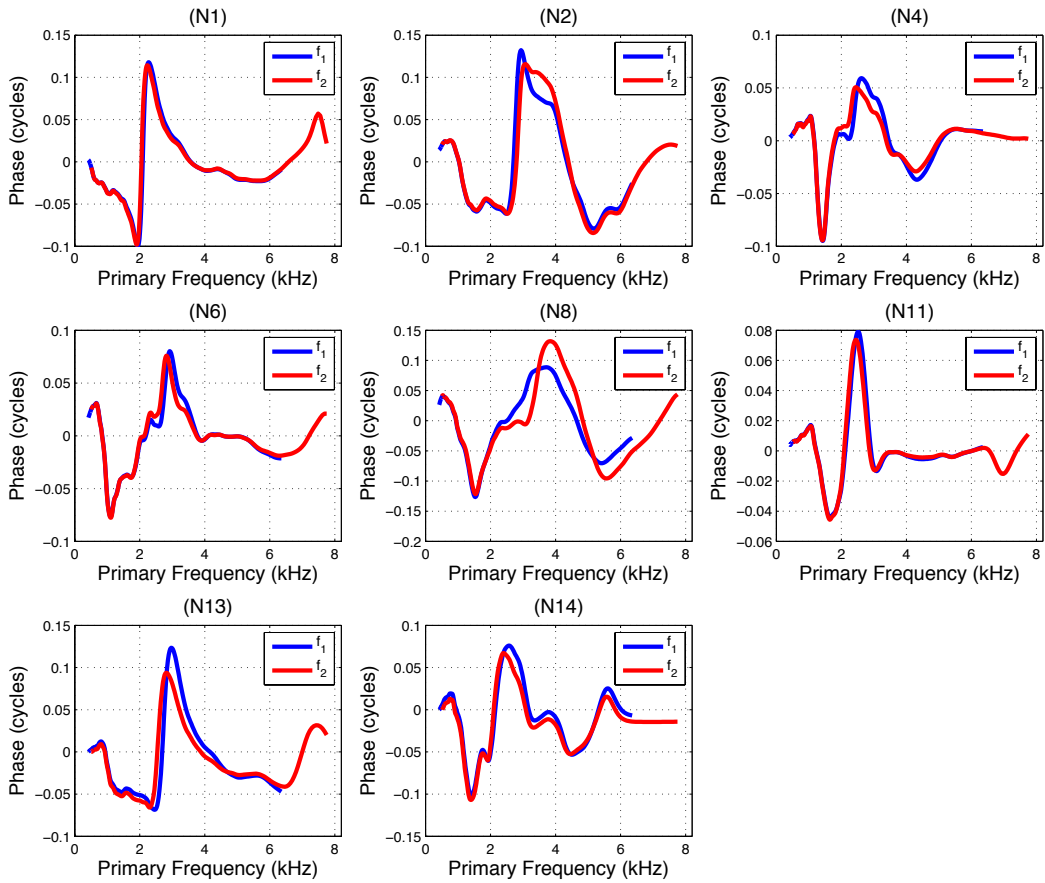


Figure 11. Phase difference (NMEP-Normal) in (cycles) for both  $f_1$  (blue) and  $f_2$  (red) at primary level L65 dB SPL. Both primaries exhibit similar patterns. Each subject has a narrow region of maximum phase difference between 2000 and 4000 Hz.

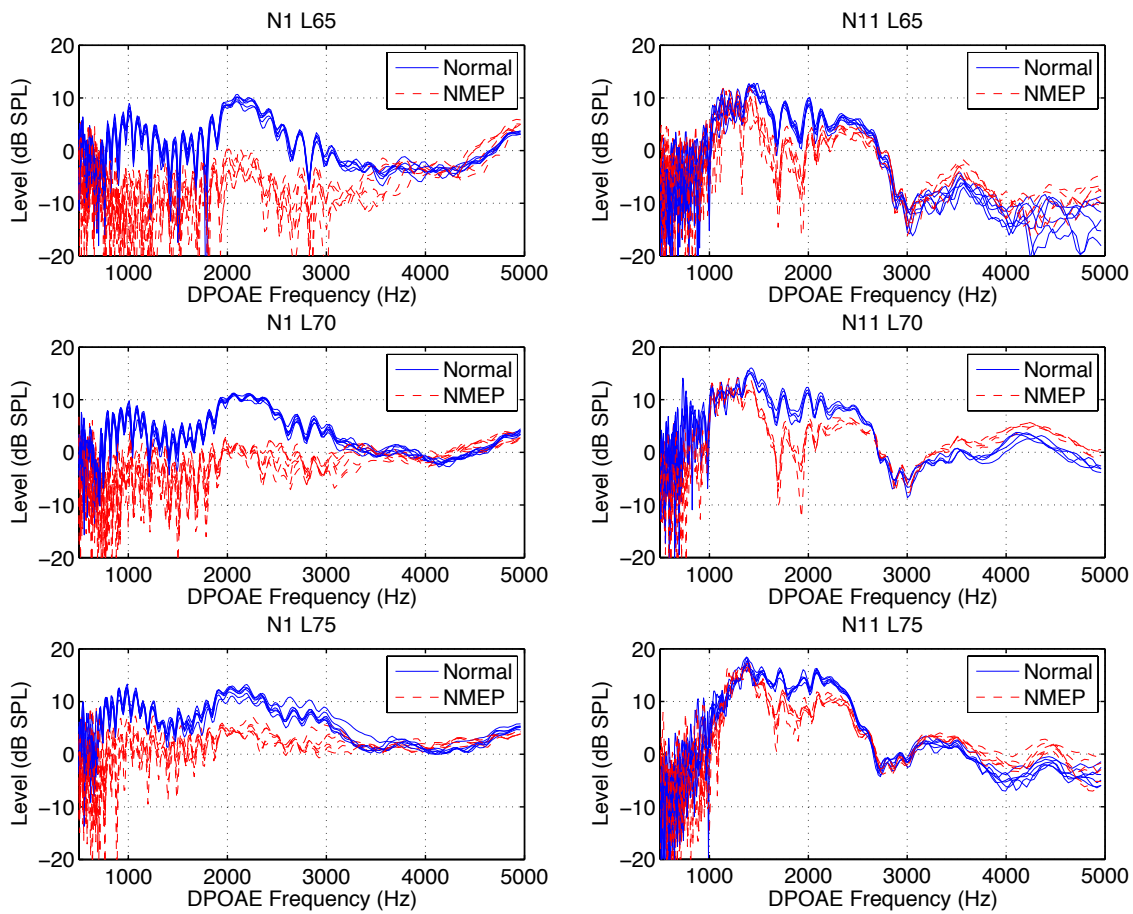
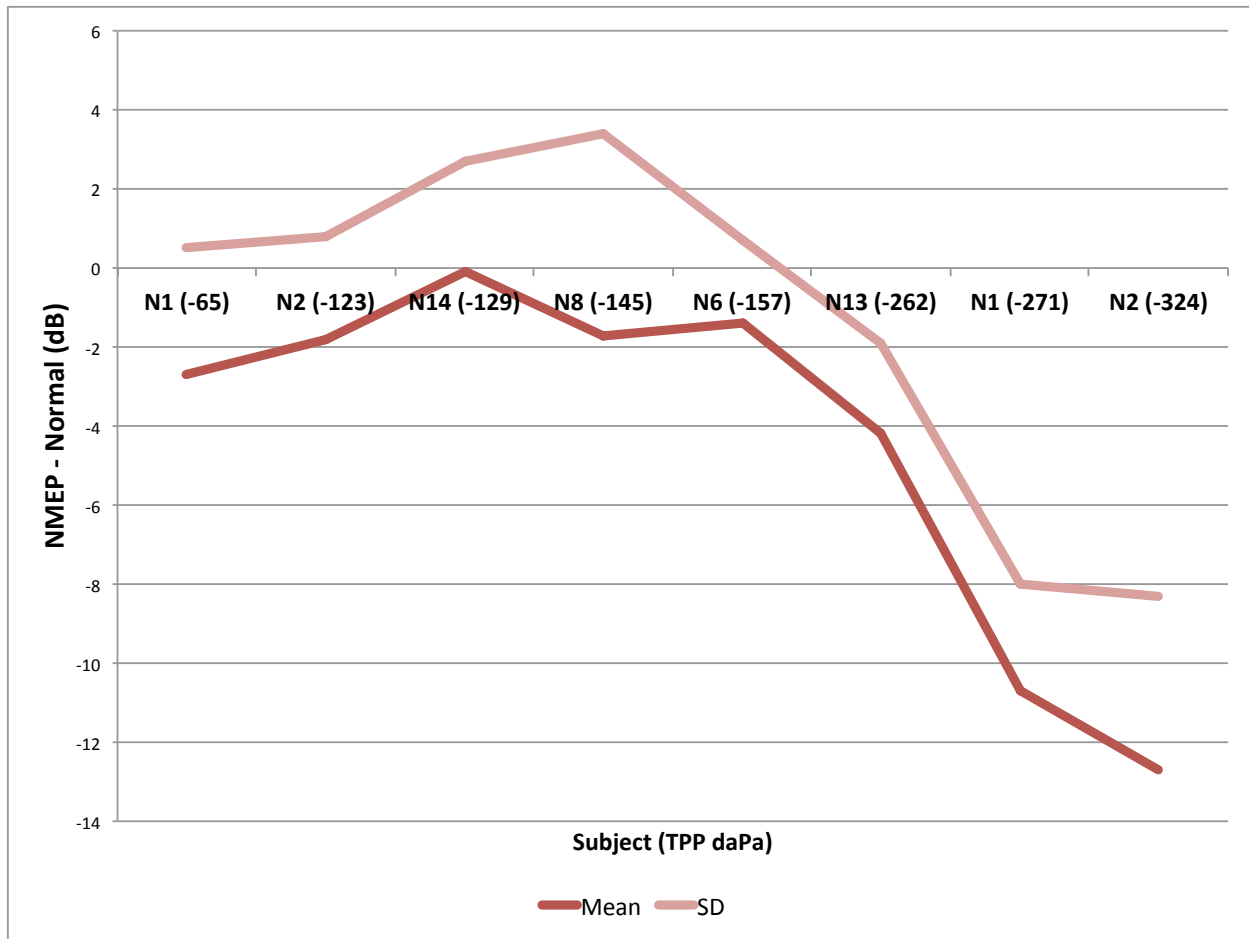


Figure 12. Composite DPOAE level as a function of DPOAE frequency at each of the three primaries tested (L65, L70, L75 dB SPL) are displayed above for subjects N1 (TPP -271 daPa) and N11 (TPP -65 daPa). Each normal middle ear trial is represented with blue lines and each NMEP trial with red dashed lines. For every subject, composite DPOAE level was decreased during NMEP for some of the frequencies tested. The patterns are subject-specific and consistent across primaries L65, L70, L75 dB SPL.



*Figure 13.* Composite DPOAE level change in decibel (NMEP – Normal) averaged across frequency at primary L65 dB SPL. Across frequency, subject N2 was most affected by NMEP. For subject N2, the composite DPOAE level was -12.7 dB lower in level across frequency during NMEP compared to Normal.

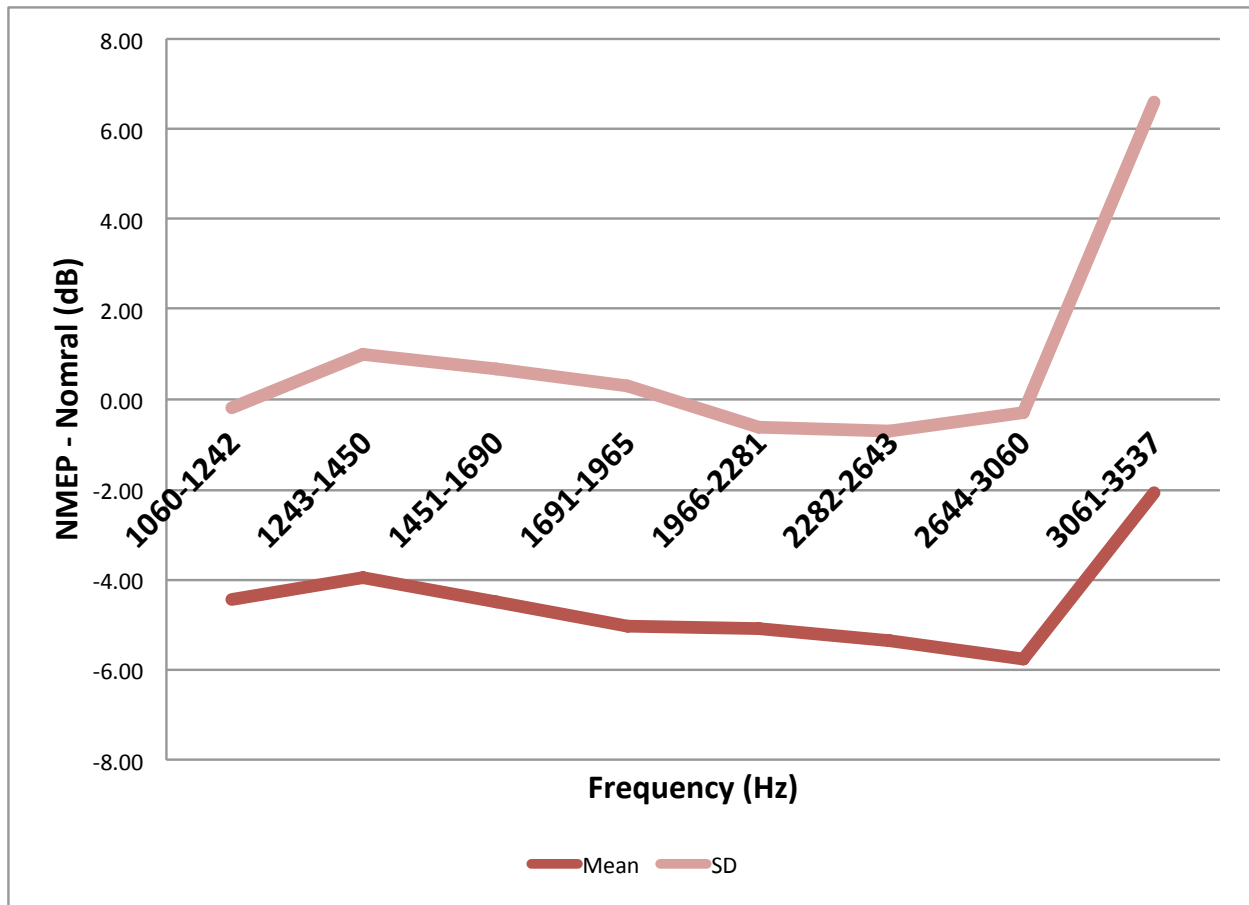


Figure 14. Mean and standard deviation composite DPOAE level changes (NMEP – Normal) across subject for primary L65 dB SPL are displayed. Data was binned by frequency according to the Greenwood function. Across subject, the frequency region between 2644 and 3060 Hz was most affected by NMEP with an average decrease in DPOAE level of -5 dB.

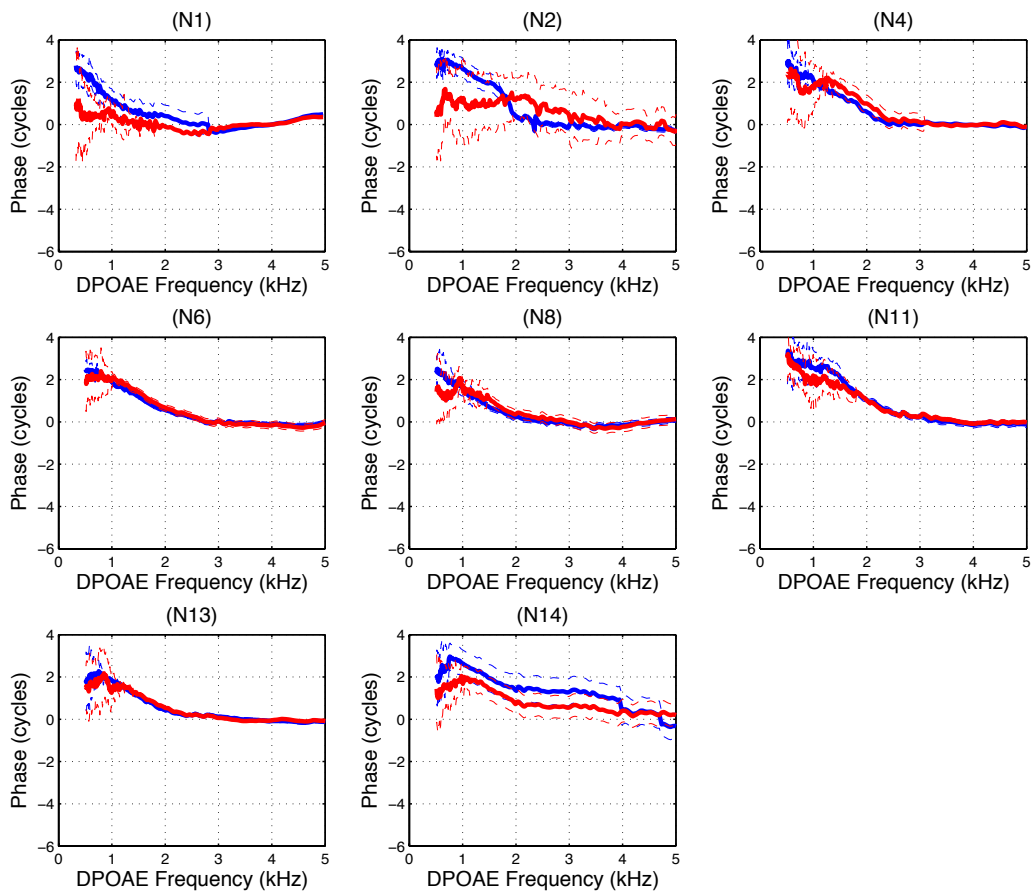
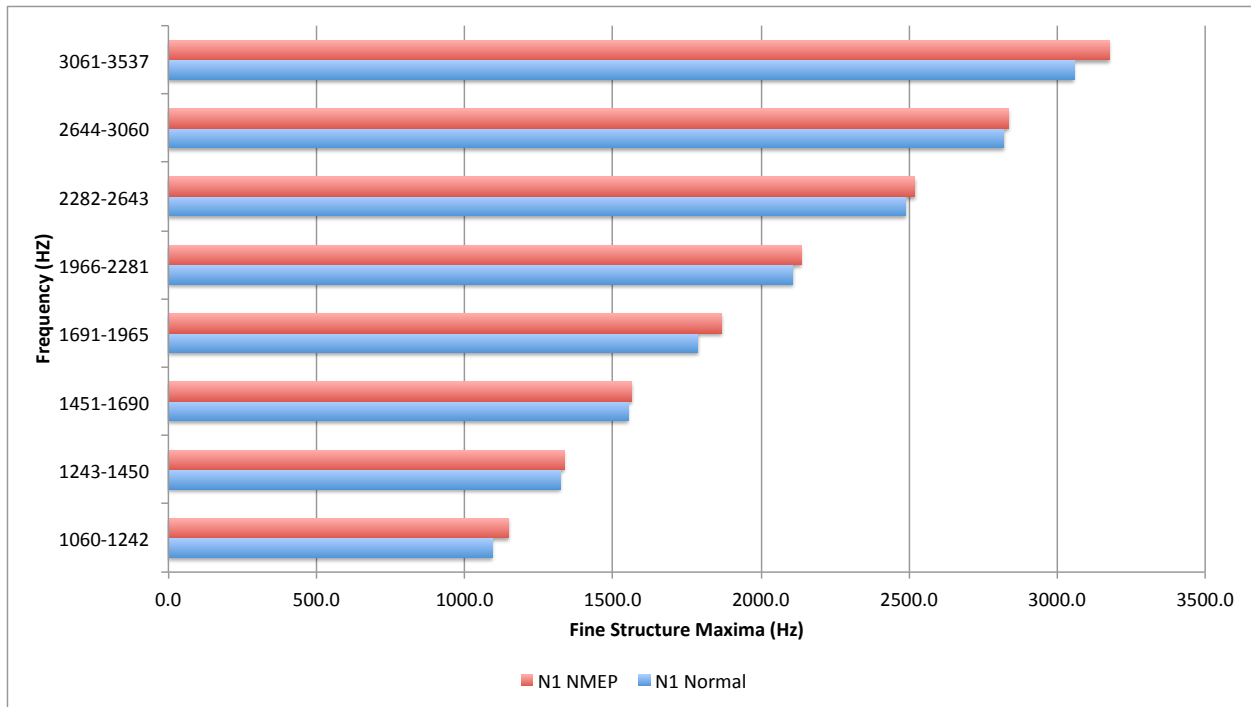
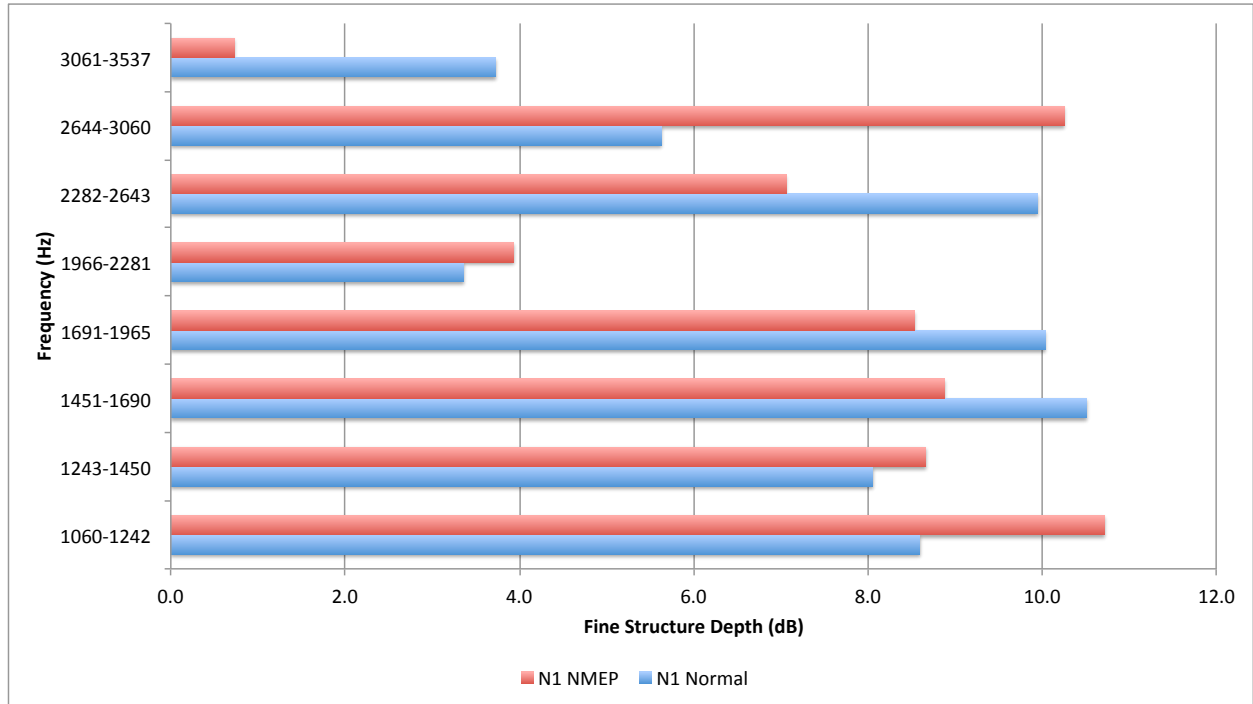


Figure 15. Composite DPOAE phase (cycles) at primary level L65 (dB SPL) for normal pressure (blue) and NMEP (red) normalized to 4000 Hz for every subject. The phase was similar for both middle ear pressures.



*Figure 16.* N1 DPOAE fine-structure frequency (Hz) maxima for NMEP (red) and normal pressure (blue) binned by frequency at primary L65 (dB SPL). There was no significant difference in DPOAE fine-structure maxima frequencies between middle ear pressures.



*Figure 17.* DPOAE fine-structure peak depths at primary level L65 (dB SPL) for NMEP (red) normal pressure (blue) binned by frequency for N1. Peak depths were calculated by subtracting points of minima from corresponding maxima. There was no significant difference in peak depths between middle ear pressures.

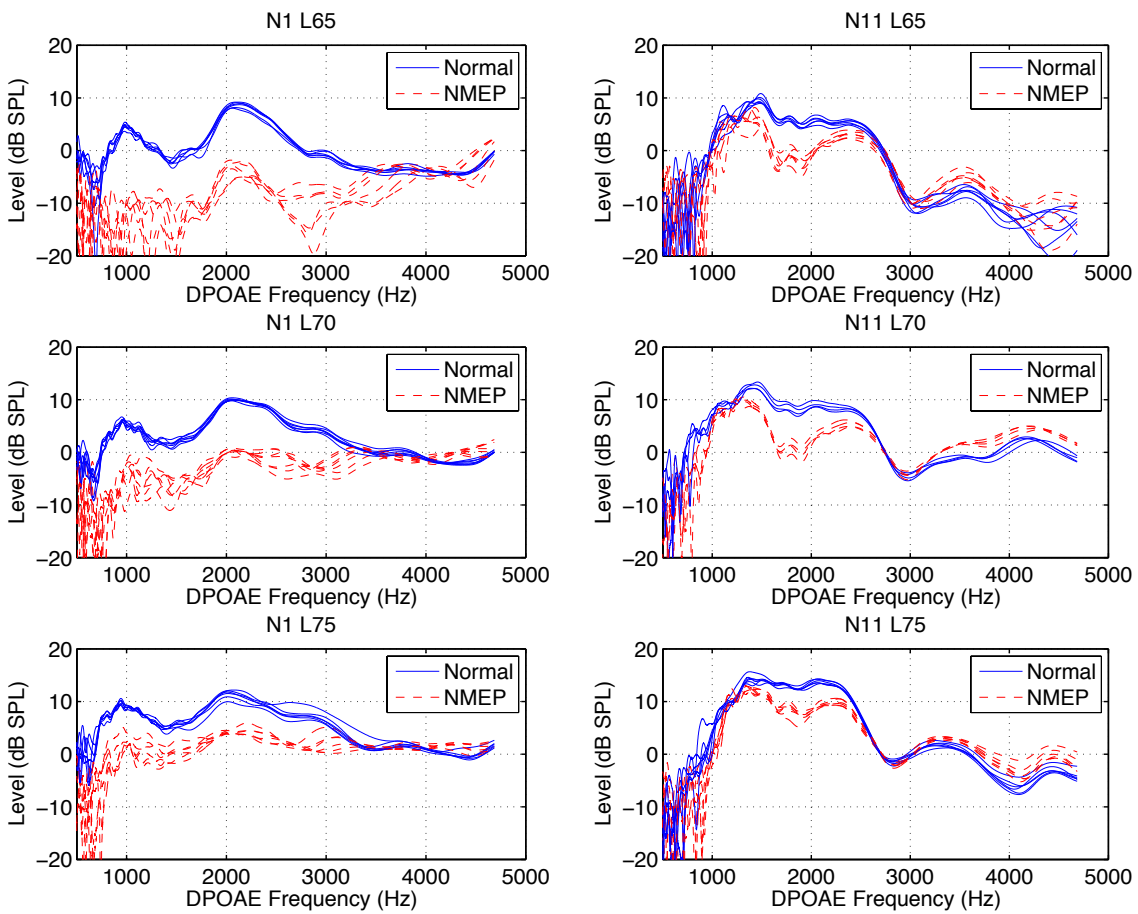


Figure 18. Generator component level (dB SPL) at the three primaries tested (L65, L70, L75 dB SPL) for subjects N1 (TPP -271 daPa) and N11 (TPP -65 daPa). Normal trials are represented with blue lines and NMEP trials with dashed red lines. Generator component data was less variable and easier to interpret than the composite DPOAE. The generator component level was lower during NMEP for all subjects at every primary tested. The pattern of change is clearer in the generator component than for the composite DPOAE.

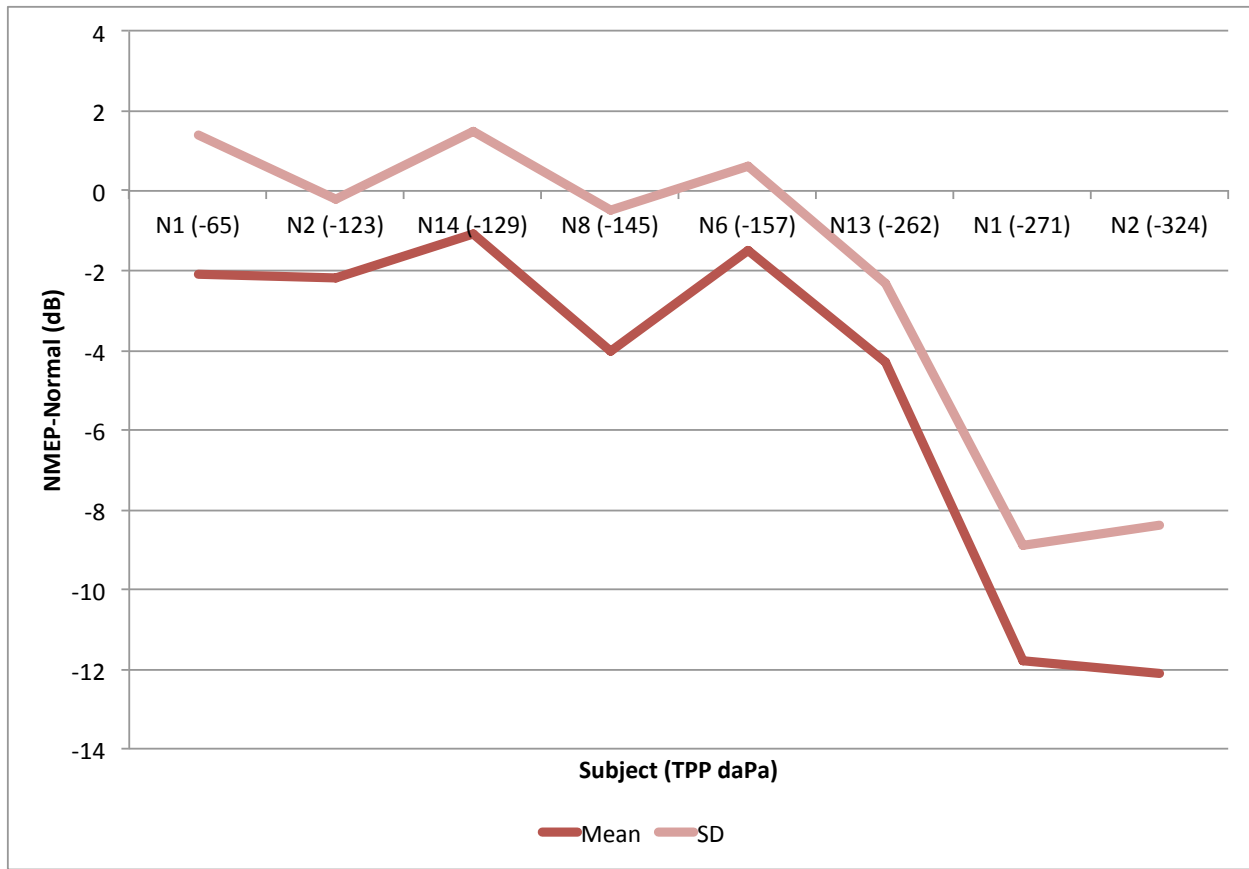
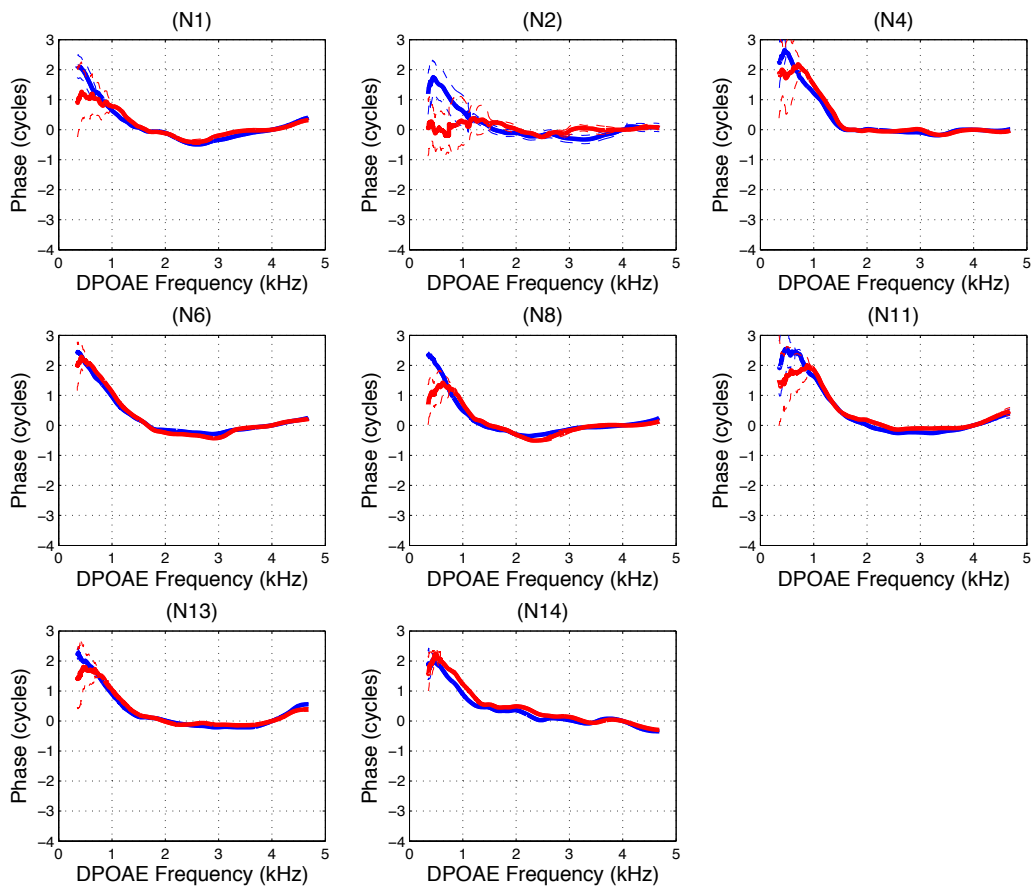
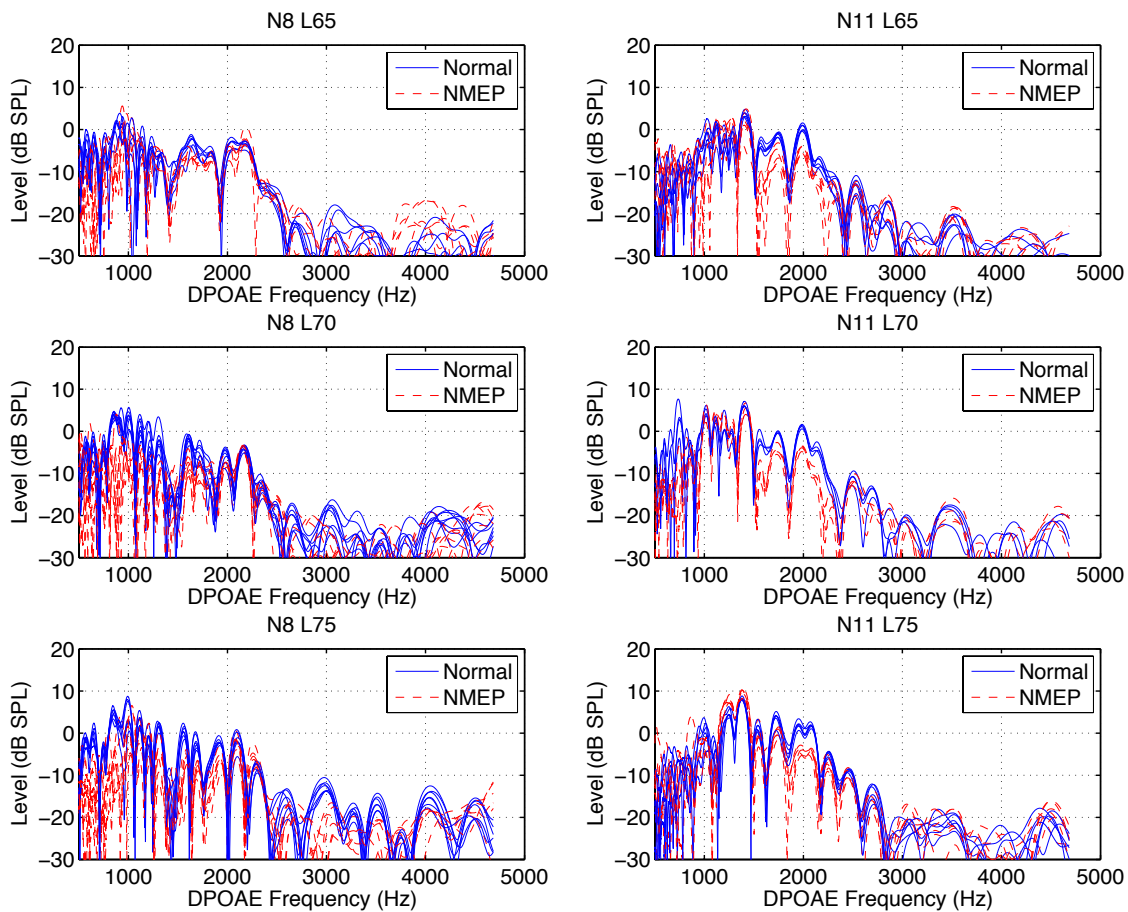


Figure 19. Mean and standard deviation generator component level difference in dB (NMEP – Normal) averaged across frequency at primary L65 dB SPL. Subject N2 was most affected by NMEP and subject N14 was least affected. More change was observed in the generator component compared to the composite DPOAE.



*Figure 20.* Generator component phase (cycles) at primary level L65 (dB SPL) for normal pressure (blue) and NMEP (red) normalized to 4000 Hz for every subject. The phase pattern of the generator component was similar to the phase of the composite DPOAE. There is little difference in phase patterns between middle ear pressures.



*Figure 21.* Reflection component level (dB SPL) at the three primaries tested (L65, L70, L75 dB SPL) for subjects N8 (TPP -145 daPa) and N11 (TPP -65 daPa). Normal trials are represented with the blue lines and NMEP trials with dashed red lines. The reflection component is lower in level and closer to the noise floor for both middle ear pressures and the difference between NMEP and Normal is less clear.

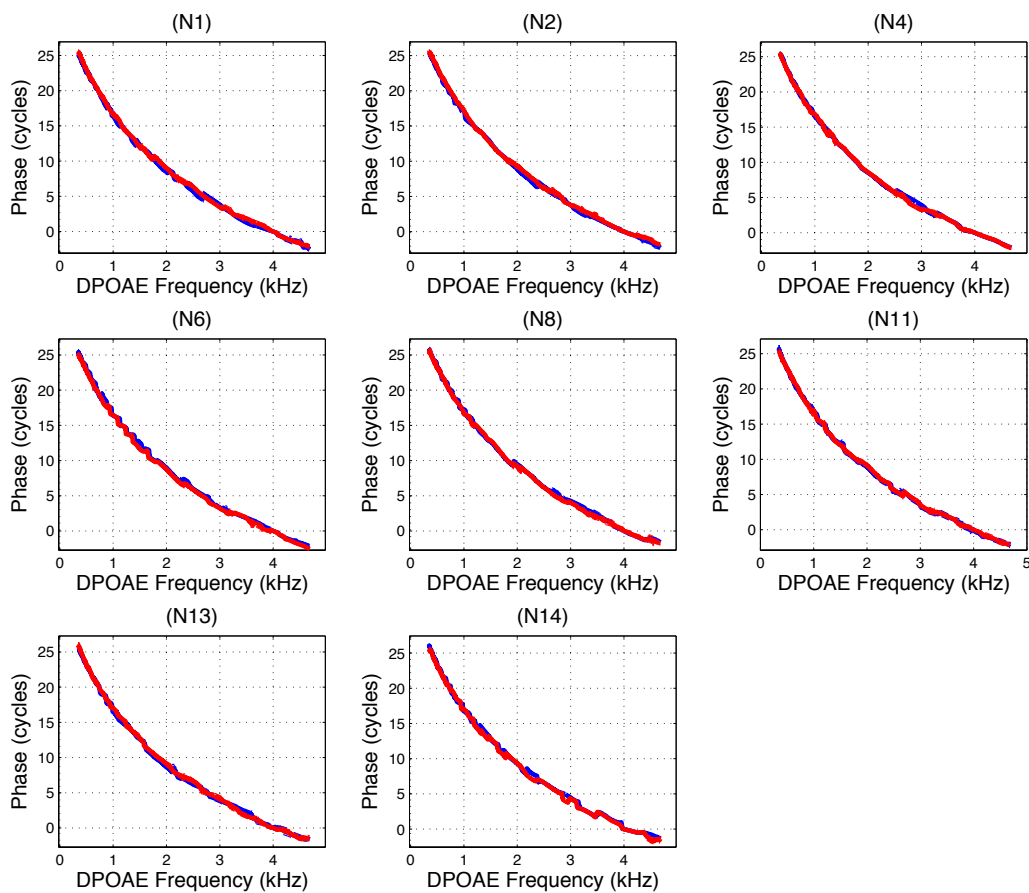


Figure 22. Reflection component phase (cycles) at primary level L65 (dB SPL) for normal pressure (blue) and NMEP (red) normalized to 4000 Hz for every subject. There is little difference in phase patterns between middle ear pressures.

## 7. Appendices

*Table 1A.* Three separate three-way repeated measures ANOVA on composite and component DPOAE level change were conducted for the effects of primary level (L65, L70, L75 dB SPL), middle ear pressure (Normal vs. NMEP) and frequency (500-4000 Hz). All effects were significant.

Composite DPOAE	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	3109.545	2	1554.772	43.152	0
Middle Ear (ME) Pressure	1616.896	1	1616.896	10.466	0.014
Frequency	4468.82	7	638.403	16.935	0
Primaries*ME Pressure	10.816	2	5.408	0.642	0.541
Primaries*Frequency	114.082	14	8.149	1.132	0.341
ME Pressure*Frequency	705.431	7	22.683	1.826	0.103
Primaries*ME Pressure*Frequency	11.429	14	0.816	0.661	0.806

Generator Component	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	3522.956	2	1761.478	57.413	0
Middle Ear (ME) Pressure	1998.411	1	1998.411	12.298	0.01
Frequency	3226.789	7	460.97	7.543	0
Primaries*ME Pressure	8.32	2	4.16	0.388	0.686
Primaries*Frequency	138.151	14	9.868	2.895	0.001
ME Pressure*Frequency	28.591	7	4.084	0.435	0.875
Primaries*ME Pressure*Frequency	7.786	14	0.566	0.747	0.722

Reflection Component	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	366.243	2	183.122	5.728	0.015
Middle Ear (ME) Pressure	723.307	1	723.307	8.191	0.024
Frequency	16846.42	7	2406.631	26.633	0
Primaries*ME Pressure	1.619	2	0.81	0.205	0.817
Primaries*Frequency	70.27	14	5.019	0.727	0.742
ME Pressure*Frequency	13.63	7	1.866	0.258	0.967
Primaries*ME Pressure*Frequency	29.981	14	2.141	1.428	0.155

Table 2A. DPOAE fine-structure maxima frequency (Hz) for normal pressure (top) and NMEP (bottom) at primary level L65 (dB SPL). Differences between points of maxima were also calculated and compared.

<b>L65 (dB SPL) Normal</b>								
	<b>1060-1242 (Hz)</b>	<b>1243-1450 (Hz)</b>	<b>1451-1690 (Hz)</b>	<b>1691-1965 (Hz)</b>	<b>1966-2281 (Hz)</b>	<b>2282-2643 (Hz)</b>	<b>2644-3060 (Hz)</b>	<b>3061-3537 (Hz)</b>
<b>N1</b>	1093.4	1323.3	1551.8	1786.9	2105.9	2489.5	2819.4	3056.9
<b>N2</b>	1145.2	1355.1	1546.3	1775.4	2045.1	2433.4	2892.6	3161.1
<b>N4</b>	1096.9	1342.1	1557.4	1803.4	2133.8	2391.6	2826.2	3175.0
<b>N6</b>	1090.0	1281.7	1507.5	1841.2	2158.1	2415.8	2835.3	3191.6
<b>N8</b>	1175.1	1349.0	1472.9	1798.5	2162.8	2385.8	2760.0	3143.9
<b>N11</b>	1202.4	1352.7	1546.3	1760.9	2069.4	2340.9	2852.2	3202.4
<b>N13</b>	1140.6	1339.6	1591.5	1867.1	2104.7	2450.0	2850.0	3129.9
<b>N14</b>	1149.0	1352.0	1607.5	1822.0	2130.9	2476.2	2939.8	3163.1
<b>Mean</b>	1136.6	1336.9	1547.6	1806.9	2113.8	2422.9	2846.9	3153.0
<b>SD</b>	40.8	24.6	42.8	35.1	41.2	49.7	52.9	45.4
<b>L65 (dB SPL) NMEP</b>								
	<b>1060-1242 (Hz)</b>	<b>1243-1450 (Hz)</b>	<b>1451-1690 (Hz)</b>	<b>1691-1965 (Hz)</b>	<b>1966-2281 (Hz)</b>	<b>2282-2643 (Hz)</b>	<b>2644-3060 (Hz)</b>	<b>3061-3537 (Hz)</b>
<b>N1</b>	1146.3	1335.4	1564.5	1865.6	2135.6	2518.7	2836.9	3175.1
<b>N2</b>	1127.4	1359.7	1590.0	1737.6	2105.0	2449.6	2856.6	3214.6
<b>N4</b>	1185.3	1334.2	1546.7	1819.0	2146.7	2478.9	2850.1	3166.7
<b>N6</b>	1147.3	1383.8	1509.8	1836.9	2158.3	2432.9	2841.9	3215.9
<b>N8</b>	1162.9	1343.8	1541.1	1870.1	2088.4	2452.9	2853.3	3094.8
<b>N11</b>	1146.2	1352.7	1544.5	1779.0	2083.0	2423.0	2795.1	3199.6
<b>N13</b>	1151.0	1350.4	1538.9	1802.0	2131.1	2476.1	2890.3	3214.2
<b>N14</b>	1154.5	1345.2	1620.2	1825.7	2138.8	2402.2	2882.3	3167.8
<b>Mean</b>	1152.6	1350.6	1557.0	1817.0	2123.4	2454.3	2850.8	3181.1
<b>SD</b>	16.6	15.9	34.2	44.1	27.8	36.6	29.2	40.8

Table 2A. DPOAE fine-structure maxima frequency (Hz) for normal pressure (top) and NMEP (bottom) at primary level L70 (dB SPL). Differences between points of maxima were also calculated and compared.

L70 (dB SPL) Normal								
	1060-1242 (Hz)	1243-1450 (Hz)	1451-1690 (Hz)	1691-1965 (Hz)	1966-2281 (Hz)	2282-2643 (Hz)	2644-3060 (Hz)	3061-3537 (Hz)
N1	1120.2	1332.5	1538.4	1823.1	2145.1	2363.5	2789.1	3190.0
N2	1123.6	1330.9	1526.5	1812.0	2115.4	2493.2	2897.6	3142.1
N4	1159.1	1317.4	1539.5	1720.0	2116.9	2353.3	2862.6	3209.7
N6	1132.4	1260.0	1490.0	1823.7	2142.7	2332.9	2816.8	3190.0
N8	1210.7	1301.2	1528.5	1769.3	2059.0	2428.9	2710.6	3111.8
N11	1159.6	1348.4	1535.5	1804.8	1991.7	2300.7	2930.3	3271.9
N13	1092.4	1290.0	1578.2	1837.6	2083.8	2386.8	3059.5	3200.0
N14	1110.9	1339.1	1583.8	1726.5	2092.3	2444.9	2841.1	3130.4
Mean	1138.6	1314.9	1540.0	1789.6	2093.4	2388.0	2863.5	3180.7
SD	37.0	29.5	29.8	45.6	50.4	63.7	103.9	51.4
L70 (dB SPL) NMEP								
	1060-1242 (Hz)	1243-1450 (Hz)	1451-1690 (Hz)	1691-1965 (Hz)	1966-2281 (Hz)	2282-2643 (Hz)	2644-3060 (Hz)	3061-3537 (Hz)
N1	1139.2	1277.1	1554.3	1793.0	2114.9	2490.1	2877.8	3250.0
N2	1142.5	1355.1	1502.8	1780.2	2087.7	2491.7	2855.5	3246.9
N4	1175.4	1377.2	1617.6	1798.6	2114.2	2372.9	2791.1	3181.4
N6	1139.0	1350.0	1516.0	1824.2	2139.8	2350.0	2665.3	3164.6
N8	1104.0	1341.1	1543.2	1784.0	2071.0	2435.1	2676.2	3216.8
N11	1157.4	1351.0	1590.0	1773.3	2071.8	2634.3	2859.0	3350.0
N13	1159.3	1345.6	1579.2	1679.3	2098.7	2433.4	2820.0	3226.9
N14	1170.8	1321.7	1591.1	1785.9	2103.2	2439.6	2839.4	3146.9
Mean	1148.4	1339.8	1561.8	1777.3	2100.2	2455.9	2798.0	3222.9
SD	22.7	29.7	39.7	42.5	23.3	87.5	82.9	63.7

Table 2A. DPOAE fine-structure maxima frequency (Hz) for normal pressure (top) and NMEP (bottom) at primary level L75 (dB SPL). Differences between points of maxima were also calculated and compared.

<b>L75 (dB SPL) Normal</b>								
	<b>1060-1242 (Hz)</b>	<b>1243-1450 (Hz)</b>	<b>1451-1690 (Hz)</b>	<b>1691-1965 (Hz)</b>	<b>1966-2281 (Hz)</b>	<b>2282-2643 (Hz)</b>	<b>2644-3060 (Hz)</b>	<b>3061-3537 (Hz)</b>
<b>N1</b>	1112.7	1339.6	1557.0	1802.5	2114.8	2490.8	2753.3	3061.0
<b>N2</b>	1168.0	1378.3	1578.0	1957.0	2170.5	2467.6	2850.4	3083.0
<b>N4</b>	1085.9	1311.5	1598.9	1731.5	2067.2	2444.4	2770	3157.6
<b>N6</b>	1127.5	1343.3	1466.9	1863.6	2196.2	2500.0	2742.2	3400.0
<b>N8</b>	1161.3	1288.4	1621.4	1855.5	2075.3	2521.6	2665.6	3068.2
<b>N11</b>	1126.1	1335.1	1529.2	1835.3	2085.1	2282.0	2933.8	3263.1
<b>N13</b>	1085.4	1288.6	1563.8	1807.4	2070.5	2346.5	3050.0	3175.0
<b>N14</b>	1155.6	1365.9	1510.0	1946.2	2135.4	2427.7	2891.2	3169.4
<b>Mean</b>	1127.8	1331.3	1553.2	1849.9	2114.4	2435.1	2832.1	3172.2
<b>SD</b>	32.3	33.1	49.8	74.9	49.0	82.2	123.9	114.3
<b>L75 (dB SPL) NMEP</b>								
	<b>1060-1242 (Hz)</b>	<b>1243-1450 (Hz)</b>	<b>1451-1690 (Hz)</b>	<b>1691-1965 (Hz)</b>	<b>1966-2281 (Hz)</b>	<b>2282-2643 (Hz)</b>	<b>2644-3060 (Hz)</b>	<b>3061-3537 (Hz)</b>
<b>N1</b>	1164.0	1350.0	1579.1	1828.1	2153.5	2502.7	2846.6	3140.0
<b>N2</b>	1148.2	1321.3	1528.9	1775.7	2079.3	2472.9	2920.6	3186.9
<b>N4</b>	1150.0	1370.2	1604.1	1743.6	2074.5	2329.2	2667.3	3150.0
<b>N6</b>	1164.6	1341.6	1477.4	1868.9	2134.6	2308.0	2740.0	3164.6
<b>N8</b>	1080.7	1373.2	1637.2	1888.8	2150.7	2411.8	2725.0	3139.1
<b>N11</b>	1201.2	1327.2	1537.8	1741.6	2092.1	2590.0	2864.7	3216.7
<b>N13</b>	1160.0	1331.5	1481.9	1820.2	2082.0	2393.3	2820.6	3287.9
<b>N14</b>	1151.9	1363.1	1580.3	1829.5	2145.2	2528.6	2894.1	3189.1
<b>Mean</b>	1152.6	1347.3	1553.3	1812.1	2114.0	2442.1	2809.9	3184.3
<b>SD</b>	33.5	20.1	57.0	54.4	35.0	98.6	89.6	49.8

Table 3A. DPOAE fine-structure peak depths at primary level L65 (dB SPL) for normal pressure (top table) and NMEP (bottom table) were calculated by subtracting points of minima from corresponding maxima.

<b>L65 (dB SPL)</b>								
<b>Peak Depth (dB) Normal</b>								
	<b>1060-1242 (Hz)</b>	<b>1243-1450 (Hz)</b>	<b>1451-1690 (Hz)</b>	<b>1691-1965 (Hz)</b>	<b>1966-2281 (Hz)</b>	<b>2282-2643 (Hz)</b>	<b>2644-3060 (Hz)</b>	<b>3061-3537 (Hz)</b>
<b>N1</b>	8.6	8.0	10.5	10.0	3.4	9.9	5.6	3.7
<b>N2</b>	2.2	14.7	12.0	9.4	7.1	7.6	1.7	7.3
<b>N4</b>	7.3	7.0	8.5	5.0	2.0	5.2	5.3	4.6
<b>N6</b>	4.0	7.9	2.8	6.1	1.2	2.5	1.7	0.7
<b>N8</b>	3.2	2.9	5.5	5.1	7.0	1.8	1.1	0.9
<b>N11</b>	2.0	2.7	9.6	7.3	4.9	8.7	6.1	0.7
<b>N13</b>	10.3	3.7	5.0	1.8	1.4	7.5	4.4	3.0
<b>N14</b>	3.0	7.3	1.6	2.4	5.5	11.6	8.0	7.1
<b>Mean</b>	5.1	6.8	6.9	5.9	4.1	6.9	4.2	3.5
<b>SD</b>	4.0	0.5	6.3	5.4	1.5	1.2	1.7	2.4
<b>L65 (dB SPL)</b>								
<b>Peak Depth (dB) NMEP</b>								
	<b>1060-1242 (Hz)</b>	<b>1243-1450 (Hz)</b>	<b>1451-1690 (Hz)</b>	<b>1691-1965 (Hz)</b>	<b>1966-2281 (Hz)</b>	<b>2282-2643 (Hz)</b>	<b>2644-3060 (Hz)</b>	<b>3061-3537 (Hz)</b>
<b>N1</b>	10.7	8.7	8.9	8.5	3.9	7.1	10.3	0.7
<b>N2</b>	5.3	5.4	6.3	5.2	7.6	10.5	6.8	4.1
<b>N4</b>	8.8	4.0	9.7	7.5	3.0	3.9	5.1	3.1
<b>N6</b>	2.8	4.4	2.6	6.5	2.3	3.0	3.1	0.7
<b>N8</b>	3.5	2.1	4.2	1.3	5.7	4.7	1.7	1.0
<b>N11</b>	1.7	6.8	13.6	10.6	3.6	1.7	8.7	0.8
<b>N13</b>	7.0	2.0	3.0	2.2	1.9	4.3	2.5	1.0
<b>N14</b>	1.5	7.0	1.4	3.1	4.5	7.0	4.6	5.0
<b>Mean</b>	5.2	5.0	6.2	5.6	4.1	5.3	5.3	2.0
<b>SD</b>	6.5	1.2	5.3	3.9	0.4	0.1	4.0	3.0

Table 3A. DPOAE fine-structure peak depths at primary level L70 (dB SPL) for normal pressure (top table) and NMEP (bottom table) were calculated by subtracting points of minima from corresponding maxima.

<b>L70 (dB SPL)</b>								
<b>Peak Depth (dB) Normal</b>								
	<b>1060-1242 (Hz)</b>	<b>1243-1450 (Hz)</b>	<b>1451-1690 (Hz)</b>	<b>1691-1965 (Hz)</b>	<b>1966-2281 (Hz)</b>	<b>2282-2643 (Hz)</b>	<b>2644-3060 (Hz)</b>	<b>3061-3537 (Hz)</b>
<b>N1</b>	6.4	4.5	3.4	2.2	1.1	6.7	3.5	1.4
<b>N2</b>	9.8	11.4	8.5	6.6	4.2	4.6	2.5	6.0
<b>N4</b>	20.0	4.5	6.1	6.3	1.2	3.6	4.9	2.9
<b>N6</b>	1.7	6.9	2.3	2.9	0.7	4.2	3.8	0.8
<b>N8</b>	1.8	2.3	2.5	1.7	3.0	1.2	2.4	1.0
<b>N11</b>	1.7	2.7	6.6	2.5	4.8	0.8	3.3	0.6
<b>N13</b>	5.9	2.4	4.4	1.7	1.1	10.1	1.0	8.9
<b>N14</b>	3.1	4.2	1.4	0.7	3.8	11.5	3.0	3.3
<b>Mean</b>	6.3	4.9	4.4	3.1	2.5	5.4	3.1	3.1
<b>SD</b>	2.3	5.8	0.7	1.0	1.9	3.4	0.4	1.9
<b>L70 (dB SPL)</b>								
<b>Peak Depth (dB) NMEP</b>								
	<b>1060-1242 (Hz)</b>	<b>1243-1450 (Hz)</b>	<b>1451-1690 (Hz)</b>	<b>1691-1965 (Hz)</b>	<b>1966-2281 (Hz)</b>	<b>2282-2643 (Hz)</b>	<b>2644-3060 (Hz)</b>	<b>3061-3537 (Hz)</b>
<b>N1</b>	5.9	5.2	8.0	7.0	3.0	2.5	2.6	0.2
<b>N2</b>	4.3	2.6	13.1	9.3	4.8	8.0	2.0	2.0
<b>N4</b>	15.5	4.2	6.0	4.3	2.8	7.0	2.2	1.7
<b>N6</b>	2.1	3.7	2.0	4.0	1.0	4.9	5.4	0.9
<b>N8</b>	3.3	2.7	2.2	2.3	3.0	2.7	4.6	0.9
<b>N11</b>	2.0	4.5	14.2	10.2	3.3	4.7	4.5	0.1
<b>N13</b>	6.8	2.3	4.5	4.6	2.0	1.2	6.9	0.8
<b>N14</b>	2.0	5.1	2.0	2.2	4.3	6.4	4.3	2.5
<b>Mean</b>	5.2	3.8	6.5	5.5	3.0	4.7	4.1	1.2
<b>SD</b>	2.8	0.1	4.3	3.4	0.9	2.7	1.2	0.4

Table 3A. DPOAE fine-structure peak depths at primary level L75 (dB SPL) for normal pressure (top table) and NMEP (bottom table) were calculated by subtracting points of minima from corresponding maxima.

L75 (dB SPL)								
Peak Depth (dB) Normal								
	1060-1242 (Hz)	1243-1450 (Hz)	1451-1690 (Hz)	1691-1965 (Hz)	1966-2281 (Hz)	2282-2643 (Hz)	2644-3060 (Hz)	3061-3537 (Hz)
N1	4.7	3.5	2.3	1.5	1.5	2.0	2.0	6.1
N2	8.1	14.2	5.6	3.6	2.3	2.7	1.3	3.3
N4	23.1	3.3	3.9	4.8	1.7	1.5	5.3	1.6
N6	3.1	2.3	4.9	1.8	3.6	0.3	4.2	1.9
N8	1.2	1.2	2.9	1.1	0.6	1.1	0.4	1.1
N11	1.2	1.9	2.9	3.6	2.3	17.2	1.2	0.6
N13	7.2	1.0	3.7	1.4	0.9	6.8	0.9	8.6
N14	3.0	3.2	2.0	2.7	4.5	9.2	2.3	4.4
Mean	6.5	3.8	3.5	2.6	2.2	5.1	2.2	3.4
SD	1.2	0.2	0.2	0.8	2.1	5.1	0.2	0.8
L75 (dB SPL)								
Peak Depth (dB) NMEP								
	1060-1242 (Hz)	1243-1450 (Hz)	1451-1690 (Hz)	1691-1965 (Hz)	1966-2281 (Hz)	2282-2643 (Hz)	2644-3060 (Hz)	3061-3537 (Hz)
N1	5.3	5.2	4.2	2.9	1.8	2.3	1.3	1.3
N2	6.5	4.5	4.8	3.3	4.1	7.1	2.6	1.3
N4	22.5	3.4	4.0	5.5	2.4	4.3	5.7	1.0
N6	1.4	2.0	5.0	1.6	0.6	3.7	5.4	1.4
N8	2.1	1.4	1.7	0.4	2.5	1.3	4.5	1.7
N11	0.7	2.4	8.8	5.6	3.6	7.0	1.1	0.8
N13	4.8	1.0	2.6	1.7	1.4	4.5	3.5	0.5
N14	3.0	3.6	1.7	3.0	4.3	13.3	2.6	4.0
Mean	5.8	2.9	4.1	3.0	2.6	5.4	3.3	1.5
SD	1.7	1.2	1.8	0.1	1.8	7.8	0.9	2.0

Table 4A. A three way repeated measures ANOVA on primary level (L65, L70, L75 dB SPL), frequency band (8 bands from 1060-3537 Hz), and middle ear pressure (Normal vs. NMEP) was performed for every subject for peak width and peak depth. There were no significant changes in peak width (top panel) or peak depth (bottom panel).

N1 Peak Width

	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	782.163	2	391.081	1.187	0.368
Frequency	31860.355	6	5310.059	1.004	0.453
Middle Ear (ME) Pressure	295.475	1	395.475	3.526	0.157
Primaries*Frequency	9559.999	12	796.667	1.191	0.326
Primaries*ME Pressure	26.841	2	13.42	0.329	0.732
Frequency*ME Pressure	2392.076	6	398.679	1.042	0.431
Primaries*Frequency*ME Pressure	7777.031	12	648.086	1.271	0.277

N1 Peak Depth

	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	72.067	2	36.033	0.215	0.812
Frequency	158.627	7	22.61	0.651	0.71
Middle Ear (ME) Pressure	2.297	1	2.297	0.111	0.761
Primaries*Frequency	206.17	14	14.726	1.057	0.421
Primaries*ME Pressure	20.833	2	10.417	0.739	0.517
Frequency*ME Pressure	130.009	7	18.573	1.456	0.236
Primaries*Frequency*ME Pressure	222.343	14	15.882	1.22	0.297

N2 Peak Width

	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	46610.093	2	23305.047	1.045	0.408
Frequency	96676.976	6	16112.829	0.957	0.481
Middle Ear (ME) Pressure	13937.572	1	13937.572	0.86	0.422
Primaries*Frequency	234017.524	12	19501.46	0.99	0.477
Primaries*ME Pressure	38821.866	2	19410.933	1.059	0.404
Frequency*ME Pressure	127217.218	6	21202.87	1.012	0.448
Primaries*Frequency*ME Pressure	235294.906	12	19607.909	0.998	0.47

N2 Peak Depth

	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	148.5	2	36.033	0.215	0.601
Frequency	178.75	7	25.536	0.703	0.67
Middle Ear (ME) Pressure	13.653	1	13.653	0.643	0.481
Primaries*Frequency	187.925	14	13.423	0.893	0.572
Primaries*ME Pressure	24.773	2	13.423	0.93	0.445
Frequency*ME Pressure	73.382	7	10.483	0.488	0.832
Primaries*Frequency*ME Pressure	202.532	14	14.467	1.024	0.45

## N4 Peak Width

	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	70.741	2	35.37	1.188	0.368
Frequency	63884.655	6	10647.443	0.981	0.466
Middle Ear (ME) Pressure	91.524	1	91.524	0.248	0.653
Primaries*Frequency	16042.748	12	1336.896	1.239	0.296
Primaries*ME Pressure	77.597	2	38.798	0.972	0.431
Frequency*ME Pressure	1177.351	6	196.225	0.583	0.739
Primaries*Frequency*ME Pressure	7822.773	12	651.898	1.21	0.314

## N4 Peak Depth

	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	237.432	2	118.716	1.067	0.401
Frequency	204.635	7	29.234	0.442	0.865
Middle Ear (ME) Pressure	4.083	1	4.083	0.204	0.682
Primaries*Frequency	275.9	14	19.707	1.137	0.356
Primaries*ME Pressure	34.77	2	17.385	1.444	0.308
Frequency*ME Pressure	72.922	7	10.417	0.718	0.658
Primaries*Frequency*ME Pressure	158.33	14	11.309	0.798	0.666

## N6 Peak Width

	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	400.636	2	200.318	1.07	0.401
Frequency	96953.165	6	16158.861	1.004	0.453
Middle Ear (ME) Pressure	367.277	1	367.277	0.447	0.552
Primaries*Frequency	15881.202	12	1323.434	1.18	0.333
Primaries*ME Pressure	351.175	2	175.587	0.404	0.684
Frequency*ME Pressure	2050.838	6	341.806	1.277	0.316
Primaries*Frequency*ME Pressure	23815.175	12	1984.598	1.248	0.29

## N6 Peak Depth

	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	203.472	2	101.736	0.868	0.467
Frequency	185.908	7	26.558	0.871	0.545
Middle Ear (ME) Pressure	4.025	1	4.025	0.201	0.684
Primaries*Frequency	235.177	14	16.798	1.263	0.27
Primaries*ME Pressure	28.202	2	14.201	0.929	0.445
Frequency*ME Pressure	94.742	6	15.79	1.04	0.432
Primaries*Frequency*ME Pressure	248.407	12	20.701	1.446	0.191

## N8 Peak Width

	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	324.151	2	162.075	2.575	0.156
Frequency	46940.225	6	7823.371	1.065	0.419
Middle Ear (ME) Pressure	1026.137	1	1026.137	1.891	0.263
Primaries*Frequency	25909.115	12	2159.093	1.127	0.37
Primaries*ME Pressure	758.317	2	379.158	1.222	0.359
Frequency*ME Pressure	6003.008	6	1000.501	1.158	0.371
Primaries*Frequency*ME Pressure	21794.481	12	1816.207	1.131	0.367

## N8 Peak Depth

	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	164.4	2	82.2	0.647	0.556
Frequency	177.141	7	25.306	0.865	0.549
Middle Ear (ME) Pressure	1.595	1	1.595	0.075	0.802
Primaries*Frequency	186.237	14	13.303	0.974	0.494
Primaries*ME Pressure	22.288	2	11.144	0.783	0.499
Frequency*ME Pressure	80.191	7	11.456	0.803	0.594
Primaries*Frequency*ME Pressure	189.912	14	13.565	1.037	0.438

## N11 Peak Width

	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	925.913	2	462.956	1.723	0.256
Frequency	87828.629	6	14638.105	0.979	0.468
Middle Ear (ME) Pressure	61.202	1	61.202	2.912	0.186
Primaries*Frequency	6995.399	12	582.95	0.883	0.571
Primaries*ME Pressure	179.56	2	89.78	0.306	0.747
Frequency*ME Pressure	51775.415	6	8629.236	0.881	0.528
Primaries*Frequency*ME Pressure	12768.653	12	1064.054	0.915	0.542

## N11 Peak Depth

	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	152.658	2	76.329	0.587	0.585
Frequency	311.325	7	44.475	1.165	0.364
Middle Ear (ME) Pressure	0.004	1	0.004	0	0.991
Primaries*Frequency	135.657	14	9.69	0.533	0.899
Primaries*ME Pressure	10.854	2	5.427	0.3	0.751
Frequency*ME Pressure	185.395	7	26.599	1.965	0.109
Primaries*Frequency*ME Pressure	111.991	14	7.999	0.496	0.922

## N13 Peak Depth

	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	301.389	2	150.694	1	0.444
Frequency	255.528	7	36.504	1	0.471
Middle Ear (ME) Pressure	4.694	1	4.694	0.158	0.729
Primaries*Frequency	243.056	14	17.361	1	0.479
Primaries*ME Pressure	34.722	2	17.361	1	0.444
Frequency*ME Pressure	121.528	7	17.361	1	0.471
Primaries*Frequency*ME Pressure	243.056	14	17.361	1	0.479

## N13 Peak Width

	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	535.877	2	267.939	2.426	0.169
Frequency	85520.359	6	14253.393	0.937	0.493
Middle Ear (ME) Pressure	173.85	1	173.85	4.899	0.114
Primaries*Frequency	11464.648	12	955.387	0.808	0.641
Primaries*ME Pressure	232.779	2	116.39	1.407	0.315
Frequency*ME Pressure	33299.71	6	5549.952	0.887	0.524
Primaries*Frequency*ME Pressure	18142.687	12	1511.891	0.892	0.562

## N14 Peak Width

	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	88.486	2	44.243	0.966	0.433
Frequency	49427.519	6	8237.92	0.932	0.496
Middle Ear (ME) Pressure	30.345	1	30.345	0.932	0.405
Primaries*Frequency	15926.894	12	1327.241	1.047	0.431
Primaries*ME Pressure	177.84	2	88.92	2.435	0.168
Frequency*ME Pressure	2451.954	6	408.659	0.812	0.574
Primaries*Frequency*ME Pressure	8555.763	12	712.98	0.831	0.619

## N14 Peak Depth

	Type III Sum of Squares	df	Mean Square	F	Sig.
Primaries	180.338	2	90.169	0.733	0.519
Frequency	448.004	7	64.001	2.288	0.067
Middle Ear (ME) Pressure	6.788	1	6.788	0.343	0.599
Primaries*Frequency	184.224	14	13.159	0.95	0.517
Primaries*ME Pressure	25.953	2	12.977	0.895	0.457
Frequency*ME Pressure	111.109	7	15.873	1.277	0.309
Primaries*Frequency*ME Pressure	231.784	14	16.556	1.326	0.234

Figure 1A. Scatter plot representing percent reflectance difference (NMEP-Normal) as a function of tympanic peak pressure for each of the 11 frequency bins. There was no correlation between degree of negativity and change in reflectance

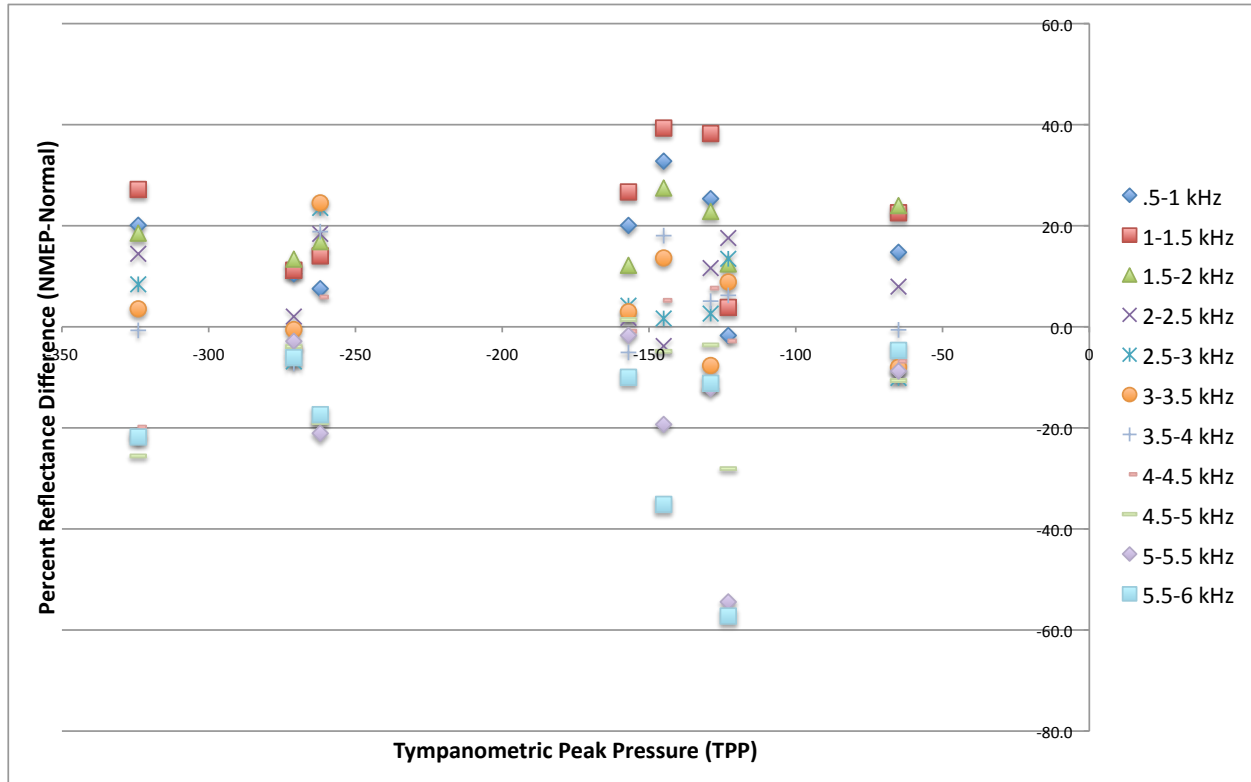
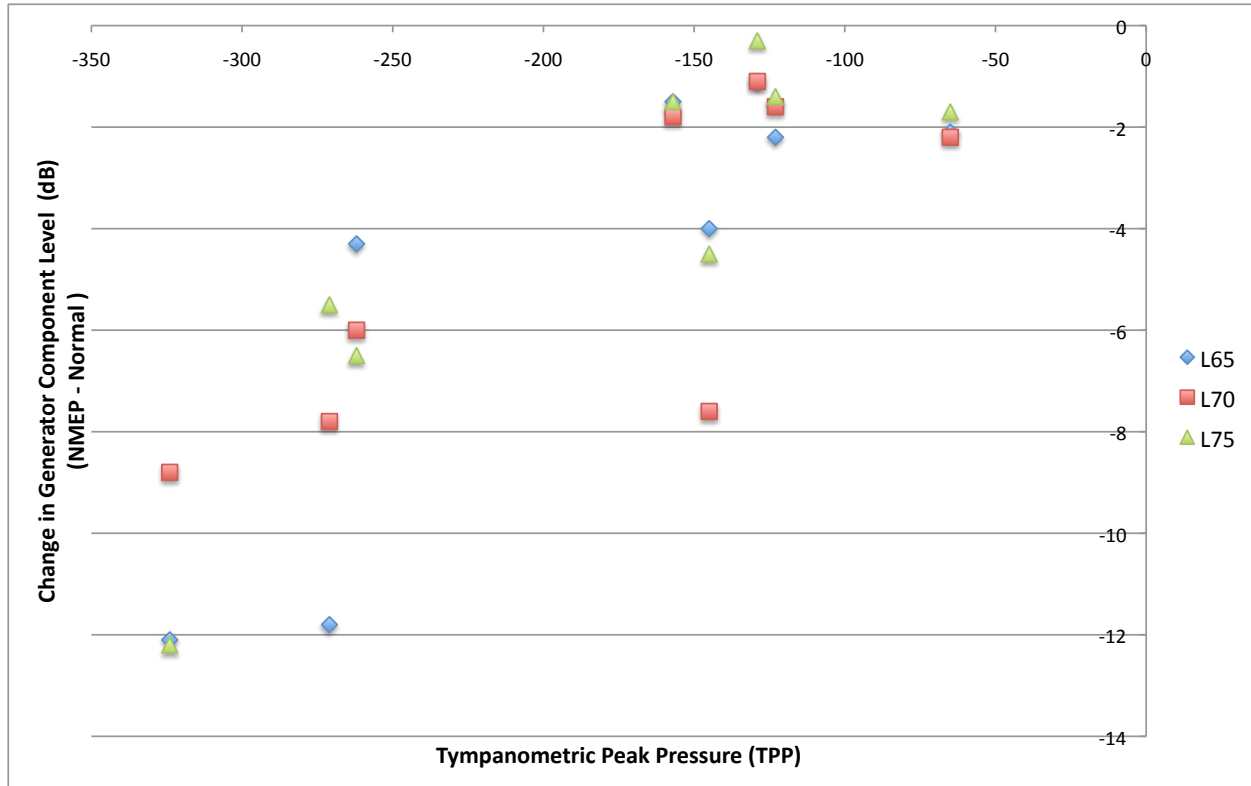


Figure 2A. Scatter plot representing generator component difference (NMEP-Normal) as a function of tympanic peak pressure for the 3 primary levels. There was a correlation between degree of negativity and change in generator component level.



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