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Effects of Carbon Dioxide and Oxygen Levels on Auditory Sensitivity and Frequency Tuning as Measured by the Stimulus Frequency Otoacoustic Emission Test

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14. ABSTRACT

Exposure to hazardous noise results in increased reactive oxygen species (ROS) activity within the cochlea that causes damage to the outer hair cells, the result is noise-induced, sensorineural hearing loss. Evoked otoacoustic emissions (EOAEs) are an electrophysiological measure of inner ear activity that reflects cochlear outer hair cell functioning during the processing of auditory stimuli. Stimulus frequency OAE (SFOAE) have the potential to assess both the sensitivity and the tuning capabilities of the cochlea. This study assessed the sub-clinical effects of increased oxygen and/or carbon dioxide levels on inner ear processing of sound as reflected by SFOAE absolute amplitude and changes in the phase of the SFOAE response as test frequencies are increased. The results of this study indicate cochlear processing is affected by breathing higher than normal levels of oxygen and carbon dioxide, but generalization of the results is limited by the small number of subjects tested. Further research is needed with a larger sample size to determine if significant changes in SFOAE amplitudes have a harmful or protective effect on cochlear functioning.

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Administrative Information

The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of the Navy, Department of Defense, or the U.S. Government. This research was conducted in compliance with all applicable federal regulations governing the protection of human subjects in research.

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ABSTRACT

Background

Exposure to hazardous noise results in increased reactive oxygen species (ROS) activity within the cochlea that causes damage to the outer hair cells. Since the outer hair cells enhance the sensitivity and tuning capabilities of the inner hair cells within the cochlea, the result is noise-induced, sensorineural hearing loss. Furthermore, increases in arterial oxygen levels will result in significantly higher oxygen levels within the cochlea. Hyperoxia results in increased oxidative metabolic activity that will in turn increase the production of oxygen free radicals or ROS in bodily tissues. Increased carbon dioxide levels also result in an increase in the amount of oxygen in body tissues through vasodilation induced increases in blood flow. Evoked otoacoustic emissions (EOAEs) are an electrophysiological measure of inner ear activity that reflects cochlear outer hair cell functioning during the processing of auditory stimuli. Stimulus frequency otoacoustic emissions (SFOAE) have the potential to assess both the sensitivity and the tuning capabilities of the cochlea. This study assessed the sub-clinical effects of increased oxygen and/or carbon dioxide levels on inner ear processing of sound as reflected by SFOAE absolute amplitude and changes in the phase of the SFOAE response (auditory tuning) as test frequencies are increased.

Methods

Initial SFOAE testing was performed to measure individual baselines and the test-retest reliability of SFOAEs using air as the control gas condition. Next, subjects underwent audiometric and SFOAE testing, with the latter test performed after exposure to air or one of several different gas mix conditions that all involved higher than normal oxygen and/or carbon dioxide levels. The following gas mix conditions high in oxygen (O_2) and carbon dioxide (CO_2) were used: 100% O_2, 94% O_2/6% CO_2, 97.5% O_2/2.5% CO_2, 21% O_2/6% CO_2, 21% O_2/2.5% CO_2, and air served as the control condition.

Results

Hyperoxic and hypercapnic gas mixes appeared to have an effect on cochlear processing of sound as reflected by single frequency SFOAE absolute amplitude measures over the range of 1957- 4043 Hz in normal hearing subjects. Among the measurable shifts from all 10 subjects, 29% single frequency SFOAE amplitude shifts exhibited significant decrements greater than 2 standard deviations of baseline amplitudes compared to 10% of significant increments for single frequencies following exposure to the different gas mixtures. SFOAE absolute amplitude was highly correlated with end tidal O_2 and CO_2 measures indicating a relationship between high levels of oxygen metabolism in the cochlea and SFOAE amplitude. This resulted in a substantial number of significant emission shifts when the ten participants were exposed to hyperoxic and hypercapnic gas mixes. The majority of significant shifts in SFOAE amplitudes were decrements, although some significant increments in amplitude occurred. SFOAE group delay (which is related to auditory tuning) did not appear to be affected by gas conditions high in oxygen and carbon dioxide.

Conclusions

The results of this study indicate cochlear processing is affected by breathing higher than normal levels of oxygen and carbon dioxide, but generalization of the results is limited by the small number of subjects tested. Further research is needed with a larger sample size to determine if significant changes in SFOAE amplitudes have a harmful or protective effect on cochlear functioning.

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INTRODUCTION

Background

Navy divers potentially are at risk for carbon dioxide toxicity during either surface supplied or self-contained breathing apparatus (SCUBA) diving operations (Lanphier & Camporesi, 1993; Lambertson, 1980). Navy special-forces divers (Sea Air Land or SEALs, Explosive Ordnance Disposal Divers or EOD) are potentially exposed to either hyperoxia (increased level of oxygen in bodily tissues) or hypercapnia (increased level of carbon dioxide in bodily tissues) when breathing 100% oxygen using closed-circuit underwater breathing apparatus. With closed-circuit rebreathing diving systems, a proportion of the diver's expiration is re-breathed after passing through a filtering system that removes carbon dioxide. After filtering, the expiration is mixed with the 100% oxygen mix from the closed-circuit system. A diver's prolonged exposure to high oxygen levels can result in pulmonary or central nervous system oxygen toxicity. Common symptoms of oxygen toxicity include throat tickle, cough, substernal burning sensation, loss of peripheral vision, nausea, facial twitching, tingling, dizziness, and seizures (Clark, 1993). Special-forces divers are at particular risk for oxygen toxicity since they commonly breathe 100% oxygen under pressure. They are also at risk for hypercapnia if the closed-circuit filtering system fails. Working divers are also at risk for hypercapnia if the air supply is unclean (e.g., intake of compressor used to fill tanks and flasks is located too close to the compressor exhaust.) Hypercapnia symptoms include unconsciousness, dizziness, rapid pulse, vasodilation, dyspnea, headache, and progressive mental confusion (Lanphier & Camporesi, 1993; Lambertson, 1980). Navy saturation, surface-supplied, and diving medical technicians are also exposed to high levels of oxygen while performing surface oxygen decompression ($SurDO_2$) or when breathing oxygen during decompression and recompression (treatment) dives.

Hyperoxia, Hypercapnia, and Reactive Oxygen Species

Hyperoxia results in increased oxidative metabolic effects that will in turn increase the production of oxygen free radicals or reactive oxygen species (ROS) in body tissues (Clark, 1993). Hypercapnia, either alone or in combination with hyperoxia, will result in a further increase in tissue oxygen levels and ROS activity due to vasodilatation effects increasing blood flow and tissue perfusion (Prazma, 1982). The potential effect of an increase in ROS (e.g., the generation of superoxide dismutase, catalase, or glutathione peroxidase in body cell tissues) is to overwhelm the body's antioxidant defense systems leading to cellular damage (Clark, 1993; Kopke et al., 2001). Breathing high concentration oxygen gas mixes will also result in increased oxidative metabolism in the inner ear, with or without accompanying hypercapnia, within time periods as short as five minutes (Prazma, 1982).

Through the same mechanism of excessive oxidative metabolism, ROS generation secondary to acoustic overexposure has been shown to damage the sensory cells of the inner ear in animal studies (Slepecky, 1986; Ohlemiller & Dugan, 1998; Kopke, et al., 1999; Kopke et al., 2001). This results in damage to the outer hair cells of the inner ear with subsequent increases in hearing threshold levels (Kopke et al., 1999, 2001; Eckenhoff et al., 1948; Prazma, 1982). Since the underlying mechanism for producing physiological changes (ROS) appears to be the same

for both acoustic and oxygen over-stimulation, it is important to examine changes in cochlear functioning associated with hyperoxia and/or hypercapnia. Navy Special Force's divers are exposed to an array of weapon and ordnance noise exposures as well as high oxygen and potentially high carbon dioxide levels using closed-circuit combat diving systems. Novel strategies and treatments, such as the use of different antioxidant combinations to repair or prevent noise damage in the inner ear are being developed and studied (Kopke et al., 1999, 2001). With the potential for applying these treatment approaches, it is important to identify operational units within the Navy and armed forces where personnel may be at greater risk for occupational, permanent hearing loss. These populations can be the first to benefit from new auditory treatment strategies.

There are little human data available regarding the effects of hyperoxia, hypercapnia, or the two in combination upon auditory sensitivity. Gellhorn and Spiesman (1935) suggested that all three gas conditions significantly increase absolute hearing thresholds. Unfortunately, the exact methodology and technical procedures used to measure absolute hearing threshold levels in their study and the statistical methods used to compare pre- and post-exposure hearing levels were not reported. Although it has been demonstrated that hypoxia does not appear to have a significant effect on hearing threshold levels (Tonndorf, 1953) or speech recognition in noise (Marshall, 1987), the effects of hyperoxia and hypercapnia on cochlear processing are not well understood.

Studies have examined the influence of c*arbogen* gas on human susceptibility to temporary threshold shift (TTS) of hearing levels secondary to acoustic overexposure (Dengerink, Miller, and Wright, 1984; Lindgren, Dengerink, and Axelsson, 1989; Chaturvedi, Rai, and Sharma, 1988; Chaturvdi, et al., 1984). Carbogen has been defined as a breathing gas that consists of 90-95% oxygen and 5-10% carbon dioxide, but typically is a 95%/5% mix respectively that results in both hyperoxic and hypercapnic conditions (Lindgren et al., 1989; Chaturvedi et al., 1988). These studies found that TTS magnitudes and recovery functions were both reduced when carbogen gas was breathed by subjects either prior to or during high level noise exposures. These potentially beneficial effects have been attributed to increased cochlear blood flow associated with hypercapnia-induced vasodilation of the cochlear artery, which results in increased oxygenation of body tissues (Dengerink et al., 1984; Lingren et al., 1989; Prazma, Rodgers, and Pillsbury, 1983). These investigators proposed that carbogen's protective effect was due to vasoconstriction of the cochlear artery with a resultant decrease in oxygenation of the cochlea (Dengerink et al., 1984).

The medial and lateral olivocochlear bundle-mediated efferent suppression of the cochlear outer hair cells during high-noise exposure has also been studied (Maison & Liberman, 2000). The results of this study suggested that individuals who are “strong OAE suppressors” show significantly less permanent threshold shift (PTS) of hearing levels than those who exhibited a “weaker” suppression effect. It was proposed that this might account for the variability that exists between individuals regarding susceptibility to noise-induced hearing loss (Maison & Liberman, 2000). Carbogen and OAE suppression studies examined noise as a stressor, but it is not clear whether hyperoxia and/or hypercapnia have a detrimental or protective effect on cochlear processing of sound. Such studies would have important implications for the Navy Special Forces, saturation, and working diving communities.

The use of traditional psychophysical methods (e.g., method of limits, method of constant stimuli, method of adjustment, two-interval forced-choice) to measure cochlear processing indirectly is time-consuming and highly dependent on subject responses. All of these methods have inherent test-retest variability due to the influence of subject, equipment, procedural, and environmental variables (Wolgemuth, Marshall, and Lapsley Miller, 2003; Lane, et al., 1985; Dobie, 1983). An additional variable would be cognitive decrements associated with breathing higher than normal carbon dioxide levels (Lanphier & Camporesi, 1993; Lambertson, 1980). Use of an objective measurement (e.g., stimulus frequency otoacoustic emissions) to study the effects of hyperoxia and hypercapnia on cochlear processing would not require behavioral responses from subjects. The goal of this study was to determine if elevated levels of oxygen (hyperoxia) and/or carbon dioxide (hypercapnia) result in a significant change in cochlear sensitivity and/or frequency tuning capabilities using a stimulus frequency otoacoustic emissions test.

Otoacoustic Emissions

The healthy inner ear produces sound while processing auditory stimuli (Kemp, 1978; Shera & Guinan, 1999). Otoacoustic emissions (OAEs) are sounds generated within a normally-functioning cochlea, either spontaneously in approximately 50% of the population (Norton & Stover, 1994) or in response to acoustic or electrical stimulation for the majority of individuals who have normal hearing sensitivity or whose hearing threshold levels do not exceed 25-30 dB HL (Kemp, 1978**;** Shera et al., 2002). The type generated by an acoustic stimulus is known as evoked otoacoustic emissions (EOAEs). There are three main methods for evoking EOAES: transient evoked otoacoustic emissions (TEOAEs), distortion-product otoacoustic emissions (DPOAEs), and stimulus-frequency otoacoustic emissions (SFOAES). Two of these types, TEOAEs and DPOAEs, have been studied extensively as potential clinical tools for detecting sub-clinical, pre-clinical, and both temporary and permanent changes in cochlear processing secondary to noise-exposure in humans (e.g., Lapsley Miller & Marshall, 2001). TEOAE and DPOAE studies have used commercial or custom-designed test systems and parameters to assess the relationship between noise exposure and otoacoustic emissions (Lapsley Miller & Marshall, 2001; Mansfield, Baghurst, and Newton, 1999; Attias, Bresloff, Reshef, Horowitz, and Furman, 1998; Sliwinska-Kowalska, 1998).

The third type of EOAE is the stimulus frequency otoacoustic emission (SFOAE) developed by Shera and Guinan (1999) and Kemp and Chum (1980). To date, the use of SFOAE measurements has been restricted to research laboratories where the emphasis has been on theory, not application. There is little to no information about the validity and reliability of SFOAEs in the literature at this time that could be used to develop this type of EOAE measurement technique for clinical usage (Lapsley Miller, Boege, Marshall, Shera, and Jeng, 2004). Validity is concerned with establishing that the SFOAE measurement consists primarily of an evoked stimulus-frequency otoacoustic emission response that is not contaminated with stimulus waveform energy via cross-talk, noise, or other measurement artifacts. Reliability is concerned with the repeatability of the SFOAE measurements over time. Shera and Guinan's method, however, has now been implemented in a commercial unit, the Mimosa Acoustics SFOAE system. SFOAEs potentially provide more frequency-specific information about the cochlea than DPOAEs or TEOAEs.

Shera and Guinan (1999) proposed that EOAEs be classified by how they are generated within the cochlea rather than by the type of external stimulus used to generate them. They proposed taxonomy based on two fundamentally different mechanisms: 1) EOAEs that arise by *linear reflection* (i.e., SFOAEs and TEOAEs at medium to low stimulus levels) and 2) those that arise by *nonlinear distortion* (i.e., DPOAEs at high stimulus levels). Linear reflection emissions are thought to be backward-traveling waves arising from the basilar membrane through linear or coherent reflection of forward traveling waves near the peak of the basilar membrane traveling wave envelope (Shera & Guinan, 1999). These linear reflections are thought to originate from preexisting *perturbations* (e.g., larger outer hair cells, greater number of outer hair cells) on certain areas of the basilar membrane) (Shera & Guinan, 1999). EOAEs that arise from nonlinear distortion (e.g., DPOAEs) occur when backward-traveling waves arise from the basilar membrane as it works to "process" two single-frequency stimuli that stimulate the basilar membrane for areas in close proximity to each other (Shera & Guinan, 1999). Both linear reflection and nonlinear distortion EOAEs have potential for increasing our understanding of cochlear processing, however, the SFOAE has the greatest potential to be frequency specific and also has potential for assessing cochlear tuning capabilities in addition to sensitivity.

SFOAE research to date has focused largely on theory regarding the generation of the response and its potential to assess cochlear tuning in a laboratory environment (e.g., Shera & Guinan, 1999, 2003; Shera et al., 2002). Recently, SFOAE test-retest reliability, as implemented on a commercial OAE system, was estimated as it may have potential for supplementing audiometry in hearing conservation programs to monitor auditory function after noise exposure (Lapsley Miller, Boege, Marshall, Shera, and Jeng, 2004). Linear-reflection EOAEs (e.g., SFOAE) may have greater potential for such monitoring, because they are thought to be more vulnerable to cochlear damage from over-exposure to hazardous noise (Shera, 2004). The SFOAE may also have potential for being more sensitive to cochlear changes induced from excess levels of oxygen and/or carbon dioxide. If the inner ear is damaged, its ability to produce SFOAEs is potentially decreased and this may be measured as a decrement in amplitude. Furthermore, SFOAEs can also be used to estimate the sharpness of the peripheral auditory filter (cochlear tuning) using techniques recently developed by Shera et al. (2002) that measure SFOAE group delay. Theoretical research indicates the SFOAE procedure appears to be a more sensitive measure in estimating the inner ear's frequency tuning capabilities than most psychophysical procedures since it measures auditory processing directly without being dependent on an individual's response (Shera & Guinan, 2002, 2003). Shera, Guinan, and Oxenham (2002) demonstrated that SFOAE estimates of the sharpness of cochlear filters were comparable to neural tuning curve data.

There is virtually nothing known about how increases in oxygen and/or carbon dioxide levels in the inner ear affect auditory sensitivity and/or tuning capabilities. Currently, we know acoustic overexposure results in an increase in ROS that will potentially damage the outer hair cells of the inner ear (Kopke et al., 1999, 2001). We do not know if an increase in ROS activity secondary to high oxygen and/or carbon dioxide levels in the inner ear causes an adverse or protective effect, however, studies using carbogen gas exposure (hyperoxic and hypercapnic conditions) suggest the potential for a protective effect (Dengerink and Axelsson, 1989; Chaturvedi, Rai, and Sharma, 1988). Since Navy Special Forces divers are exposed to both noise stressors on land as well as high oxygen and potentially high carbon dioxide levels using closed-circuit combat

diving systems, it is important to obtain information regarding adverse or protective effects on cochlear functioning.

The objectives of this study were to determine if elevated levels of oxygen and/or carbon dioxide would result in a significant change in cochlear processing as reflected in SFOAE amplitude and/or frequency tuning measurements in human subjects across a portion of the speech frequency range. These goals were accomplished using a physiological measurement of inner ear functioning that did not require a response from the subject. A non-commercial version of the stimulus frequency otoacoustic emission procedure developed by investigators at the Naval Submarine Medical Research Laboratory, Massachusetts Eye and Ear Infirmary, and Mimosa Acoustics, Inc. (Lapsley Miller, Boege, Marshall, Shera, and Jeng, 2004; Shera et al., 2003; Shera et al., 2002; Shera & Guinan, 1999) was used.

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METHODS

Participants

The participants consisted of five active duty U.S. Navy divers, three active duty non-divers, one civilian U.S. Navy diver, and one civilian non-diver employee (N = 10). All subjects volunteered to participate in this project and read and signed informed consent forms approved by the Naval Submarine Medical Research Laboratory Institutional Review Board (IRB). Participants were all male and ranged in age from 28 to 48 years of age (M = 36.8; SD = 6.1 years).

Participant Screening

All participants were non-smokers or had been smoke-free as a minimum the same number of years that they had been smokers. To meet study inclusion criteria, all participants were required to have hearing threshold levels 20 dB HL or better for the test frequencies of 250 through 3000 Hz and no worse than 25 dB HL at 4000-8000 Hz in both ears. Audiometric testing was performed using a Madsen AC-40 two-channel clinical audiometer calibrated to ANSI S3.6 (1996) standards. All audiometric testing was performed in a double-walled audiometric chamber meeting ANSI S3.1 (1991) standards. Otoscopic examinations were performed to ensure ear canals were free of cerumen accumulation and that there were no external ear pathologies prior to any testing. Participants were also required to exhibit normal tympanometry results in both ears, defined as eardrum compliance between 0.3 and 1.6 ml and middle ear air pressure within ±50 daPa. Tympanometry was performed using a Grason-Stadler GS-33 Middle Ear Analyzer.

Participants underwent SFOAE testing and had to exhibit response amplitudes of at least +10 dB SNR with rapidly changing, unwrapped SFOAE phase as a function of frequency, for at least two-thirds of the test frequencies over the range of 1957-4043 Hz. Meeting these criteria allowed for measurement of emission shifts while remaining sufficiently above biological and acoustical noise floors. Cerumen removal was performed when necessary prior to any SFOAE measurements.

SFOAE Method

Participants were evaluated using the Mimosa Acoustics SFOAE measurement system, which is based on the method developed by Shera and Guinan (1999). Data collection was controlled using an IBM ThinkPad T20 laptop computer, a Starkey DP2000 PCMCIA card, with custom SFOAE software (v.1.2.2c), probe interface cable, and an Etymotics ER-10C aural probe system with low-noise microphone and receivers. The SFOAE software v.1.2.2c was a slightly earlier version of the software used by Lapsley Miller et al. (2004). Testing involved presenting pure tone stimuli in the participant's ear canal via a foam earplug attached to the ER-10C probe. The stimulus signal travels from the probe down the ear canal to the tympanic membrane, through the middle ear system, and into the cochlea. The SFOAE response is generated from a region in the cochlea near the maximum peak of the incoming traveling wave and then returns via the same

pathway as the incoming stimulus tone. The ER-10C low noise microphone measures the sound pressure in the ear canal.

The raw unprocessed signal detected by the probe is made up of three components. The largest component being the stimuli composed of a single frequency probe tone alone alternated with a probe tone plus a single frequency suppressor tone. This component was received directly from the microphones for probe channels 1 and 2 respectively. A second component is the reflection of those stimulus frequencies from the tympanic membrane. The third component is the SFOAE response generated by the outer hair cells of the basilar membrane at the region of the probe stimulus frequency. To extract the SFOAE response from the ear canal signal level, the vector difference between the probe alone and probe plus suppressor levels was obtained. This difference represented the SFOAE response (Shera & Guinan, 1999; Shera et al, 2002). The vector difference is a pure tone signal with the same frequency as the probe stimulus. The phase of the vector-derived signal differs from the phase of the original probe stimulus frequency because of the addition of a delay in SFOAE signals traveling from the cochlea (Shera et al., 2002).

The suppressor signal frequency was 47 Hz higher than the probe frequency and the probe/suppressor levels were 40/55 dB SPL, respectively. These parameters were selected as they were shown in a similar study to yield valid and reliable data for SFOAE testing (Lapsley Miller et al., 2004). Probe stimulus frequencies over the range of 1957 to 4043 Hz were used with sampling occurring in two buffers (probe stimulus alone and probe stimulus plus suppressor tone buffers) until a total of 32 artifact-free otoacoustic emission responses were obtained. The sampling rate was 48 kHz and the FFT size was 4096 points. The Mimosa Acoustics system measured the noise floor for both probe stimulus alone and for the probe/suppressor stimulus. The average of these two values was taken to represent the noise floor, which is 3 dB lower than the total noise floor used in Lapsley Miller et al. (2004).

Three center frequencies were chosen (2004, 3000, and 3996 Hz) as they represented a frequency range that has been shown to be susceptible to the effects of noise (Gasaway, 1985) and are critical for speech understanding (Schow & Nerbonne, 2002; Fucci & Lass, 1999). Clusters of five measurements were made around each center frequency. A cluster consisted of two test frequencies below the center frequency, one at the center frequency, and two above the center frequency. There was a separation of 23 Hz between frequencies in a cluster and this was sufficient to accurately measure estimates of group delay at each center frequency (Lapsley Miller et al., 2004).

SFOAE group delay was also measured to estimate the auditory-filter bandwidth. Group delay is defined as the negative slope of the unwrapped phase as a function of probe-stimulus frequency (Shera & Guinan, 1999; Lapsley Miller et al., 2004). If SFOAE phase spectrum is mapped out in fine detail, it will have a steep negative slope because the auditory filter introduces a delay—the sharper the filter, the longer the delay (as measured in milliseconds) of the SFOAE response in a healthy cochlea (Shera & Guinan, 2003; Shera, Guinan, & Oxenham, 2002). Unwrapping phase corrects the phase angles in a vector by adding multiples of ±360° when absolute jumps between consecutive elements of the vector are greater than or equal to the default jump tolerance of 180°. Using simple linear regression and following the same procedures as Lapsley Miller et al.

(2004), group delay was calculated as the slope of unwrapped phase as a function of frequency for each 5-frequency cluster. Since group delay is a slope, it cannot be estimated from just one datum and therefore at least three valid data points were needed to calculate the slope.

Calibration of SFOAE System

The SFOAE system was calibrated in-the-ear using a chirp stimulus. The procedures used were similar to those of Lapsley Miller et al. (2004). The main purpose of calibration was to obtain data about the acoustic performance of the transducer and the external ear canal of the participant that would be used to help extract the SFOAE response. A second purpose was to determine whether the fitting and refitting of the probe in an individual's ear canal throughout all SFOAE testing resulted in the maintenance of constant sound pressure levels at the tympanic membrane across all measurement frequencies. The two stimulus channels were each calibrated using 50 averages.

Each stimulus buffer consisted of two sub averages: two probe-alone and two probe + suppressor intervals. The stimulus buffer was presented 16 times (16 averages), and the results of the 2x16=32 averages were analyzed as described by Shera & Guinan (1999). The noise-rejection level was at 36 dB SPL. Measurements exceeding this noise-rejection level were not collected.

Gas Conditions and Gas Delivery System

Six different gas mix conditions were used and are shown in Table 1. One of the gas conditions was room air, which functioned as the control condition. The other five gas conditions were chosen to induce different degrees of hyperoxia, hyperoxic hypercapnia, and normoxic hypercapnia. The premixed experimental gas mixes (± 0.01% tolerance) were stored in K bottles. The K bottles were secured to the floor of the double-walled sound-treated room used for SFOAE testing. Prior to testing participants, gas mix percentages were checked using an oxygen analyzer (S-3A/l Applied Electrochemistry, Pittsburgh, PA) and a carbon dioxide gas analyzer (CD-3A, Applied Electrochemistry, Pittsburgh, PA). The test gas for each subject was provided from two Douglas Bags that were filled with either air or one of the premixed gas mixtures. Subjects breathed from the Douglas bags, with their nose occluded by a nose clip and through a mouthpiece connected to a Hans Rudolph two-way valve using low resistance respiratory tubing.

The Hans-Rudolph mouthpiece and Douglas Bag system were chosen to minimize respiration noises during testing since the SFOAE response is one of low acoustic amplitude. A gas sample line was connected to the mouthpiece so that inspired and end tidal oxygen and carbon dioxide levels could be continuously monitored during testing using the aforementioned oxygen and carbon dioxide analyzers. During SFOAE testing, output from the oxygen and carbon dioxide analyzers was sampled and saved at 60 Hz by a desktop computer fitted with an analog-to-digital converter (model DAS-16F, Keithley/Metrabyte, Taunton, MA). An in-house software program was then used to analyze the end tidal carbon dioxide levels and convert them into text files. Participants were instructed to breathe normally in a relaxed manner during all gas mix administration and SFOAE testing. A bottle of pure oxygen was kept in the test room at all times to be used for medical treatment in the event that a subject experienced anxiety from breathing higher than normal CO_2 levels. The principal investigator and a research assistant were in the

room at all times during gas administration and testing. To protect the health of the subjects, if end-tidal carbon dioxide reached 9%, testing would immediately cease, since at this level and above there is a high probability of adverse symptoms (e.g., anxiety, unconsciousness).

Table 1. Gas Conditions

Name	Type	Gas Mix
Air*	Normoxia	21% oxygen/0.04% carbon dioxide
Air & low CO_2	Normoxic and Low Level Hypercapnia	21% oxygen/2.5% carbon dioxide
Air & high CO_2	Normoxic and High Level Hypercapnia	21% oxygen/6% carbon dioxide
Oxygen	Hyperoxia	100% oxygen
O_2 & low CO_2	Hyperoxic and Low Level Hypercapnia	97.5% oxygen/2.5% carbon dioxide
O_2 & high CO_2	Hyperoxic and High Level Hypercapnia	94% oxygen/6% carbon dioxide

*The air condition was used during session 2 to establish SFOAE baselines and during sessions 3 and 4 where it was interspersed among the experimental gas conditions to serve as a control condition on experimental days.

Test Sessions

There were five test sessions per participant. During session 1 (participant qualification session), audiometry was performed using the modified Hughson-Westlake procedure (ANSI S3.21, 1996). An SFOAE test was performed at six different frequencies for at least two cluster frequencies. This session typically lasted 30-40 minutes.

During session 2, participants underwent 10 repeated SFOAE tests with the examiner removing and inserting the probe between each test. Prior to SFOAE testing, an otoscopic examination and tympanometry were performed to ensure there was no excessive cerumen or abnormally negative or positive middle ear air pressure (reduced tympanic membrane compliance). If cerumen was noted in the test ear, it was removed and the participant rescheduled a few days later for SFOAE testing. The purpose of this session was to measure baseline SFOAE amplitudes, test-retest reliability, and phase values for each individual participant while breathing room air. Three participants did not meet SNR criteria for the 3996 Hz cluster frequencies so they underwent testing only for the 2004 and 3000 Hz clusters. The remaining seven participants underwent testing at the 3000 and 3996 Hz clusters. During this baseline testing, subjects breathed room air and used the same respiratory set up as for the experimental testing (including the nose clip) sessions. These test-retest reliability measures will be referred to as “baseline measures” throughout the remainder of this report. This session typically lasted 60 minutes.

Sessions 3 and 4 were the two experimental sessions. During each of these sessions, the participant was exposed to 3 of the 6 gas conditions. Otoscopic examination and tympanometry were again performed prior to testing. The participant was exposed to each gas condition for five minutes and then two SFOAE tests were performed while they continued to breathe that particular gas condition. SFOAE tests, depending on the participant’s noise rejection rate, typically took 3-4 minutes. After completion of the two SFOAE tests, the individual was taken

off the Douglas Bag and went back to breathing room air. To determine if any residual gas mix effects remained after removing the regulator, two additional SFOAE tests were performed immediately after the participant resumed breathing room air, unless it was the air condition. Tests performed after exposure to air in experimental sessions will be referred to as “air” measures throughout the remainder of this report. Subjects were given 15 minute breaks between gas conditions, including the air condition, and left the sound room during that time. The 15 minute breaks between gas exposures and the random assignment of gas mix presentations (including the air condition) were done to compensate for the fact that the gas mix order could not be fully counter-balanced with only 10 subjects. Each subject received the gas mix exposures (including the air condition) in a different order and subjects were blinded to the gas mix condition they were breathing. When the air condition was tested, individuals breathed room air through the Douglas Bag breathing system following the same procedures as for the experimental conditions, except that the respiratory hose attached to the mouthpiece was not attached to a Douglas Bag. These two sessions typically lasted 90 minutes to two hours.

Session 5 consisted of the same procedures and tests as were performed during session 1 and served as post-experimental audiometry, tympanometry, and SFOAE evaluations. Audiometry results were compared to the pre-experimental threshold levels using the Navy's significant threshold shift criteria ($\geq$ 10 dB HL average shift for 2000, 3000, and 4000 Hz, either ear). Tympanometry results were compared to clinical norms and SFOAE amplitude and phase results were compared to the participant's mean data obtained in session 2.

SFOAE Missing Data, Data Reduction, and Validity

Accounting for all 5 sessions, a total of 34 tests (files) including 10 frequencies (510, 1700, and 1190 SFOAE responses for clusters 2000, 3000, and 4000 respectively) were expected for each of the 10 subjects equaling a total of 3400 responses. All but 2 out of the 10 participants had some missing responses. Due to missing data, only 3189/3400 (94%) of the expected SFOAE responses were available to be screened for content and criterion validity. Six separate plots were done of SFOAE amplitude as a function of frequency in order to search for outliers and trends in the data. All data points that were identified as outliers were checked with the logbook by date and time to confirm that they were correctly labeled by gas condition and subject. Prior to any hypotheses testing, methods used by Lapsley Miller et al. (2004), but for version 2.1.18 of the SFOAE software, were considered in establishing the present study’s SFOAE response and group delay validity criteria.

SFOAE Single Frequency

To be accepted as a valid single frequency SFOAE response, several criteria had to be met. Specifically, the stimulus levels of the probe (probe alone interval and probe + suppressor interval) and suppressor (probe + suppressor interval) had to individually be within ±3 dB SPL of their target levels of 40 dB SPL (for probe) and 55 dB SPL (for suppressor). The difference between the probe stimulus level at the probe + suppressor interval and the suppressor stimulus level had to be within ±3 dB of the expected 15 dB SPL difference. The noise floor average (average of probe alone interval noise floor and the probe + suppressor interval noise floor)

needed to be < -15 dB SPL. In addition, there had to be 32 sub-measures averaged for each SFOAE response.

Figure 1 shows the maximum, mean and minimum SFOAE amplitudes with both noise floors plotted by single frequency grouped by cluster for all 10 participants before the SFOAE-to-noise ratio (SNR) criterion was applied (all other aforementioned criteria have been applied). The figure shows some overlap between the mean noise floors and the lowest SFOAE responses illustrating the need to apply a SNR criterion.

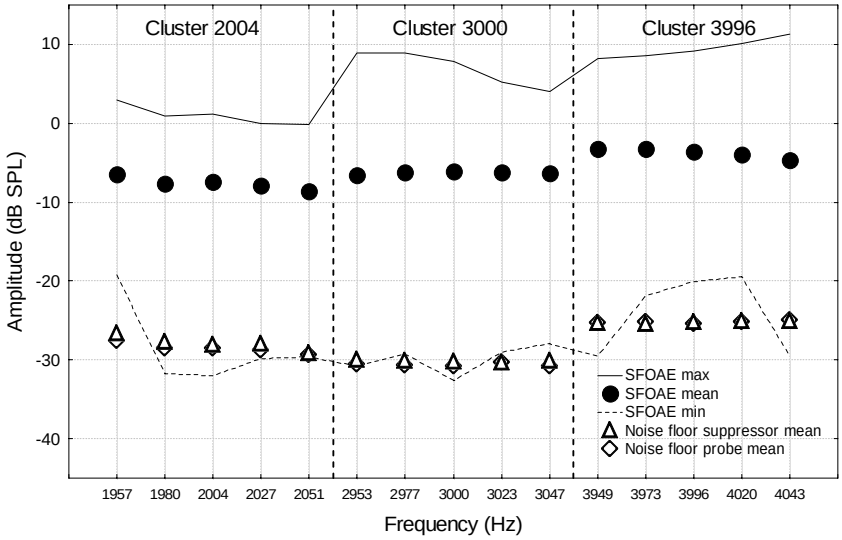

Figure 1. Maximum, mean, and minimum SFOAE amplitudes and mean probe and suppressor noise floors.

To assist in determining where to set the SFOAE-to-noise ratio (SNR) criterion, Figure 2 shows the percentage of single frequency SFOAE responses that met the aforementioned criterion as a function of SNR criterion. All single frequency measurements are clustered by their center frequency. The percentage denominators are the number of measurements collected within each cluster and the numerators are the number of measurements that met the criteria outlined above. Figure 2 shows that as SNR criterion increases the percentage of measurable SFOAE responses decrease. Because no cluster has all measurements meeting all criteria, no cluster met the 100% level for SNR even for criteria as low as –10 dB SPL. However, most measurements met the criteria from –10 dB SPL to 5 dB SPL where the percentages start to gradually decrease and this is most marked from 10 dB SPL on. Since the 3000 Hz cluster frequencies had the most measurements, its percentage is the least affected by the quantity of measurements meeting criterion. Based on these findings the minimum SNR was set at +10 dB SPL.

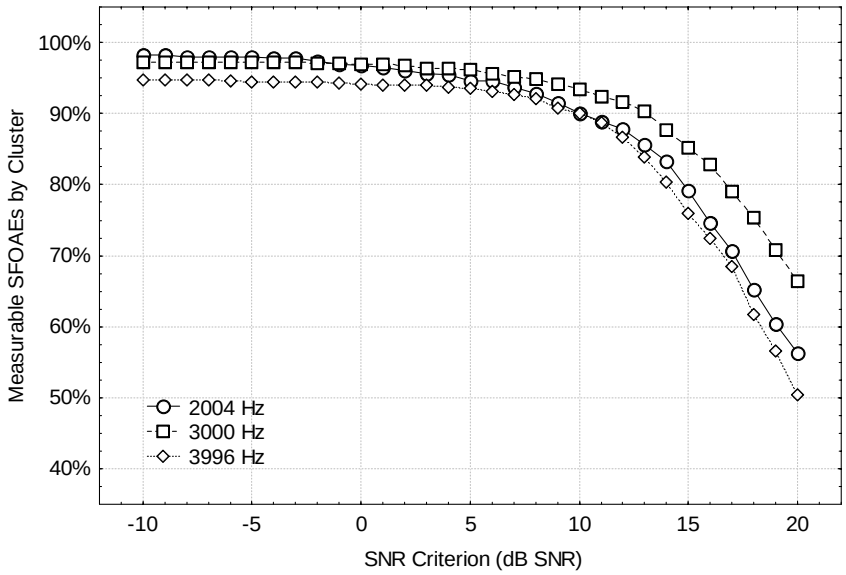

Figure 2. Percentage of valid single frequency SFOAEs grouped by cluster as a function of SNR.

Of the 3,189 available SFOAE measurements (from all SFOAE tests performed), a total of 91 (3%) did not meet the 3 dB probe stimulus criterion (outside the 37-43 dB SPL range) and 79 (2%) did not meet suppressor stimulus criterion (outside the 52-58 dB SPL range). There were 4 (< 1%) SFOAEs that did not meet the probe/suppressor 15 dB SPL difference criterion and 11 (< 1%) that did not meet the sub-measure criterion. All noise floor averages were less than -15 dB SPL. A total of 152 (5%) SFOAE responses did not meet the required 10 dB SNR. The final count of available single frequency SFOAE responses that met all criteria was 2933/3189 (92%). (It should be noted that some responses failed to meet more than 1 criterion).

SFOAE Group Delay

If all expected measurements were available there would be 102, 340, and 238 clusters for 2000, 3000, and 4000 Hz, respectively. But in order to calculate group delay at least 3 good measurements are needed from each cluster. Because of data reduction due to missing or invalid data (based on criteria), and the requirement of at least 3 SFOAE responses per cluster, the percent of "good" clusters available were 81%, 89%, and 87% for 2004, 3000, and 3996 Hz, respectively. Most good clusters included all 5 frequencies measured suggesting that if one response within a cluster was invalid (for whatever reason) the other frequencies within that cluster were also likely to be invalid. Two percent of the clusters (14/593) included 3 frequencies, 8% (47/593) included 4 frequencies, and 90% (532/593) included all 5 frequencies.

Only the data included in the "good" clusters mentioned above, were used in the group delay calculations. It was expected that across each frequency range within each cluster, as frequency increased, unwrapped phase would decrease and there would be a strong negative correlation between the two measures. If the correlation was not strong, the validity of the SFOAE measurements was believed to be poor (Lapsley Miller, Boege, Marshall, Shera, and Jeng,

2004). Furthermore, if the standard error of group delay was large, the measurement was also considered poor (Shera & Guinan, 2002). Using least squares linear regression, the slope (*b*) of the regression line for each good cluster was calculated treating frequency (Hz) as the predictor and unwrapped phase of the SFOAE in cycles as the criterion. To quantify the precision of the group delay, the standard error of the slope (*SEb*) in milliseconds (ms) (i.e., the standard error of group delay) was also calculated. Additionally, Pearson correlations of frequency and unwrapped phase were done.

Across all tests, clusters, and subjects, 66% of the correlations calculated were equal to –1.00; 97% were less than – .94; and 98% were less than – .90. Most (9/12 = 75%) of the correlations greater than –.90, included only 3 or 4 frequencies. Two of these clusters had positive slopes with slope error greater than 2 ms and correlations of .11 and .66. Including these 2 clusters, a total of 5 clusters had errors greater than 2 ms. Of these 5 clusters, correlations ranged from – .88 to .66. All clusters with group delay standard error greater than 2 ms included only 3 or 4 frequencies in the clusters.

Based on these results, clusters that included only 3-4 frequencies that had *SEb* < 2 ms and a negative and strong correlation (in this context strong is < -.90) were considered to have valid group delays. A look at the summary statistics of the group delays revealed 3 clusters that included all 5 frequencies, had low error (< 2 ms), but had correlations in the –.87 to –.90 range. To determine whether or not to keep these questionable group delays, a close examination of the data for each of these clusters was done to search for anything unusual that would not have been found using the other data criteria methods already applied. This examination revealed no further reason to exclude these clusters. Thus, in addition to the previously applied criteria for single frequencies and clusters, the following good group delay criteria were used:

- The Pearson correlation (between unwrapped SFOAE phase amplitude and frequency) had to be negative and the standard error of group delay (*SEb*) had to be < 2 ms
- If only 3 or 4 frequencies were included in the cluster, the Pearson correlation had to be < -.90
- If 5 frequencies were included in the cluster, then the Pearson had to be < -.87

It should be noted that Lapsley Miller et al. (2004) used a group delay criterion of -.94 to distinguish valid SFOAEs from invalid SFOAEs using data from normal hearing ears and from severely hearing impaired ears, which did not produce OAEs.

Applying this group delay criteria, 2% (11/593) of good clusters were excluded. This affected 50 single frequency measurements; however, group delay was only used as a criterion when clusters (SFOAE averages across cluster) were analyzed. No single frequency analyses were affected by this data reduction method.

SFOAE Reliability and Significant Shifts

EOAE amplitudes are known to vary substantially across individuals (Lapsley Miller & Marshall, 2001). Specifically, SFOAEs have been shown to exhibit variability within and across subjects that is comparable to TEOAEs (Marshall & Heller, 1998) and DPOAEs (Lapsley Miller

et al., 2004). For this reason, participants served as their own controls in order to measure significant individual changes in SFOAE amplitude or group delay after exposure to hyperoxia and/or hypercapnia gas conditions. Measuring the reliability of each participant's SFOAEs estimated the inherent level of his variability from one test to the next.

For each subject, ten SFOAE tests were conducted during session 2 and were used to calculate baseline SFOAE amplitude means and intrasubject standard deviations for single frequencies. Baseline measures for group delay were also obtained at that time. Intrasubject reliability measures were used to determine significant shifts of SFOAEs for single frequencies and group delay. For example, if an experimental gas mix single frequency, SFOAE amplitude (average of 2 gas mix exposure tests) was beyond the mean of the baseline SFOAE amplitude (± 2 intrasubject standard deviations) it was considered to be a significant emission shift (SES). If many shifts occurred, goodness-of-fit chi-square tests were done to determine if the proportion of significant shifts were significantly more than expected for single frequencies and group delay**.**

Mean Group SFOAE Amplitudes

Although the primary interest was with measuring an individual's SFOAE amplitude differences under varying gas mix conditions, whether or not the group's mean baseline SFOAE amplitudes differed from the experimental gas mix conditions was also examined. Due to the small number of subjects tested at the 2004 and 3996 Hz clusters (n = 3 and 7 respectively) this analysis only includes the 3000 Hz cluster. By individual and gas mix, valid amplitudes within this cluster were averaged yielding 1 amplitude (N = 10). Also, subject 9 did not have a valid measurement for the hyperoxia 100% O_2 gas mix condition. Rather than excluding subject 9 and running the analysis of variance test with only 9 subjects, it was decided to exclude 100% O_2 gas mix condition instead, as it showed the least promise in detecting any mean significant shifts based on its number of individual significant emission shifts found, and running the ANOVA with 10 subjects. This tradeoff was unfortunate; however, it did appear that a larger group would lend itself to a more precise determination on whether or not the experimental gas mix SFOAE amplitudes were different from the SFOAE baseline air amplitudes. To determine if mean experimental SFOAE amplitudes differed from mean baseline amplitudes, a one-way repeated measures analysis of variance for fixed effects was done. Assumptions of normality were met and the Levene and Brown-Forsythe tests of homogeneity of variance showed this assumption was also met.

SFOAEs and End Tidal Carbon Dioxide Levels

Inspired O_2 and CO_2 levels and end tidal (expired) measurements were monitored continuously during testing. Unfortunately, due to a procedural error, only 69/120 (6 conditions tested 2 times by 10 subjects) of these respiratory data files were available for comparison to the SFOAE amplitude gas mix responses. These remaining ($F_{ET}CO_2$) files were matched to their respective SFOAE data files by time, date, and experimental condition. Because the 3000 Hz SFOAE cluster frequencies (2953, 2977, 3000, 3023, and 3047 Hz) had the most available valid data out of the three clusters tested and all subjects were tested at these frequencies, only the 3000 Hz cluster was compared to the $F_{ET}CO_2$ levels . When files were matched, 3/69 respiratory files did

not match-up to good SFOAE data (as specified in the aforementioned criteria guidelines) and 1 case of an incorrect target level was also discovered. Therefore, the final data set used to examine the relationship between SFOAE and end tidal volume was reduced to 65 matched records that included 8 out of the 10 subjects. When confirming that the gas mix percentages delivered to participants were very close to target levels the 3000 Hz SFOAE cluster was also used.

Initially, mean cluster SFOAE amplitudes (3000 Hz) versus end tidal carbon dioxide levels ($F_{ET}CO_2$) were plotted determine if elevated CO_2 levels are related to SFOAEs. Preliminary examination of this plot showed two groupings of SFOAEs; those less than -5 dB SPL and those greater than -5 dB SPL. To further determine the nature of this relationship, both linear (the 2 SFOAE amplitude groups were modeled separately) and nonlinear (piecewise regression or spline) models were compared including inspired CO_2 level (%), breathing frequency rate, and $F_{ET}CO_2$ as predictors of SFOAE amplitude. For the nonlinear piecewise models, change points for SFOAE amplitude were considered in the -4.0 to -6.0 range. Regression assumptions for normality, homscedasticity, and independence of residuals, as well as model fit were checked. Model comparisons were done and final model selection was based on the percent of variability (R^2) in the SFOAE amplitude that could be explained by the model, and the significance levels and standard errors for the coefficients of each variable entered into the model.

Statistical analyses were done using SPSS for Windows (2002) and Statistica (StatSoft, 2005). Type I error probability acceptance was set at .05 for all significance tests.

RESULTS

SFOAE Reliability for Individuals at Single Frequencies

The intrasubject standard deviations that were calculated for each subject at each of their test frequencies are shown in Figure 3. These are the standard deviations that were used in determining single frequency SES. For each participant, at each frequency, a minimum of three valid SFOAE amplitudes were used to calculate each *SD*. Subjects 4, 5, and 8 were tested at the 2004 & 3000 Hz clusters, and subjects 1, 3, 6, 7, 9, 10 and 11 were tested at the 3000 and 3996 Hz clusters (there was no subject 2). Test frequencies used for each subject were dependent on SFOAE amplitude response criteria being met (as determined during session 2). Figure 3 shows that SFOAE amplitude for subjects 7 and 11 had some of the highest variability within each frequency and also varied the most across the frequencies measured. For subject 7 this could be attributed to the small number of amplitudes (based on data inclusion criteria) used to calculate his intrasubject standard deviations. In contrast, subjects 1, 3, and 9 had at least 7 valid amplitudes for each frequency and varied the least. Table 2 shows the number of amplitudes that were used to calculate all single frequency intrasubject standard deviations from the baseline air tests. Figure 3 shows the individual subject single frequency reliability.

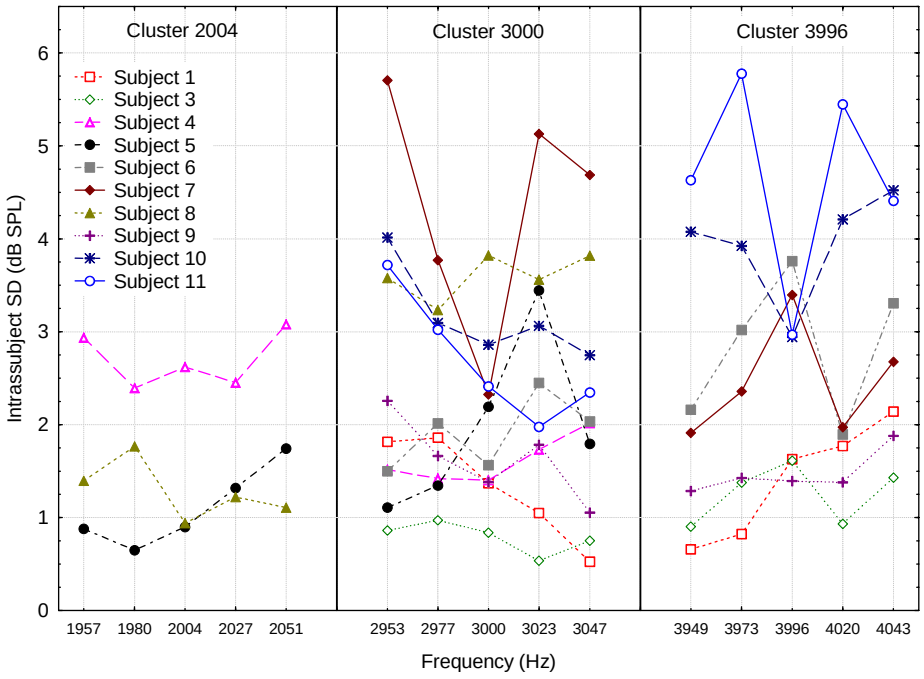

Figure 3. Individual single frequency reliability.
These are the intrasubject standard deviations for each subject.

Table 2. Number of Amplitudes Used to Calculate Intrasubject Standard Deviations

	Subject									
Frequency (Hz)	1	3	4	5	6	7	8	9	10	11
1957	*	*	9	10	*	*	8	*	*	*
1980	*	*	9	10	*	*	8	*	*	*
2004	*	*	10	10	*	*	8	*	*	*
2027	*	*	10	10	*	*	8	*	*	*
2051	*	*	10	10	*	*	7	*	*	*
2953	10	10	10	9	9	5	10	10	10	8
2977	9	10	10	9	9	4	10	10	10	8
3000	8	10	10	9	9	4	10	10	9	8
3023	7	10	10	9	8	6	10	9	9	8
3047	7	10	10	7	9	6	10	9	9	8
3949	8	10	*	*	10	8	*	9	3	9
3973	8	10	*	*	7	8	*	9	3	9
3996	8	10	*	*	10	7	*	9	6	8
4020	8	10	*	*	10	7	*	10	7	9
4043	7	10	*	*	10	7	*	10	7	9

Note. Standard deviations are shown in Figure 3. *Subject was not tested at this frequency.

Significant SFOAE Shifts – Individuals

The intrasubject standard deviations calculated for each participant for single frequency SFOAEs (Figure 3) were applied to each individual's measured SFOAE amplitudes obtained during exposure to the experimental gas mix conditions. Goodness-of-fit chi-square tests were done to determine if the proportion of shifts exceeding mean baseline ±2 *SD* were beyond what would be expected given a normal distribution.

Table 3 shows the proportion of single frequency SFOAE shifts that occurred under all gas mix conditions. Decrements are the number of SFOAE amplitudes for each gas mix significantly below the baseline, and increments are the number of amplitudes for each gas mix significantly above the baseline. From the number of shifts that occurred, the odds of having a decrement were 3 times those of an increment occurring. Decrements ranged from 12-38% per gas condition. Only the hyperoxic and lower hypercapnia conditions combined (e.g., 97% O_2/2.5% CO_2) showed a significant ($p < .001$) proportion of SFOAE increments (19%), while the other hyperoxia and/or hypercapnia conditions showed only significant decrements. With regard to which subjects showed changes, Figure 4 shows the absolute deltas for individuals from the baseline SFOAE amplitudes to those measured during the experimental conditions (baseline - gas mix) for the 3000 Hz mean cluster. This figure shows that subjects 8, 10 and 6 exhibited SES increments for all conditions while subjects 5 and 11 showed changes in both directions and the

remaining subjects showed only decrements. Among all the subjects, subject 1 appears to have been affected the most by the experimental gas mixes.

Table 3. Significant Emission Shifts from Baseline for Single Frequencies

		Decrement		Increment	
Gas-Mix	n		X^2		X^2
Air	98	12%	10.33**	3%	0.84
Air and low CO_2	100	20%	47.37***	8%	1.89
Air and high CO_2	95	29%	111.68***	8%	1.90
O_2	98	29%	111.49***	4%	0.21
O_2 and low CO_2	99	38%	229.39***	9%	3.37
O_2 and high CO_2	97	27%	92.99***	19%	35.63***

Note. Degrees of freedom = 1. Some data are missing due to data criteria restrictions. Conditions during experimental day sessions 3 and 4. Includes all single frequencies. Shifts are significantly different from baseline. Because 5% of shifts are expected to be beyond 2 SD, all expected counts for n ranging from 95-100 are 5.
**p < .01. ***p < .001.

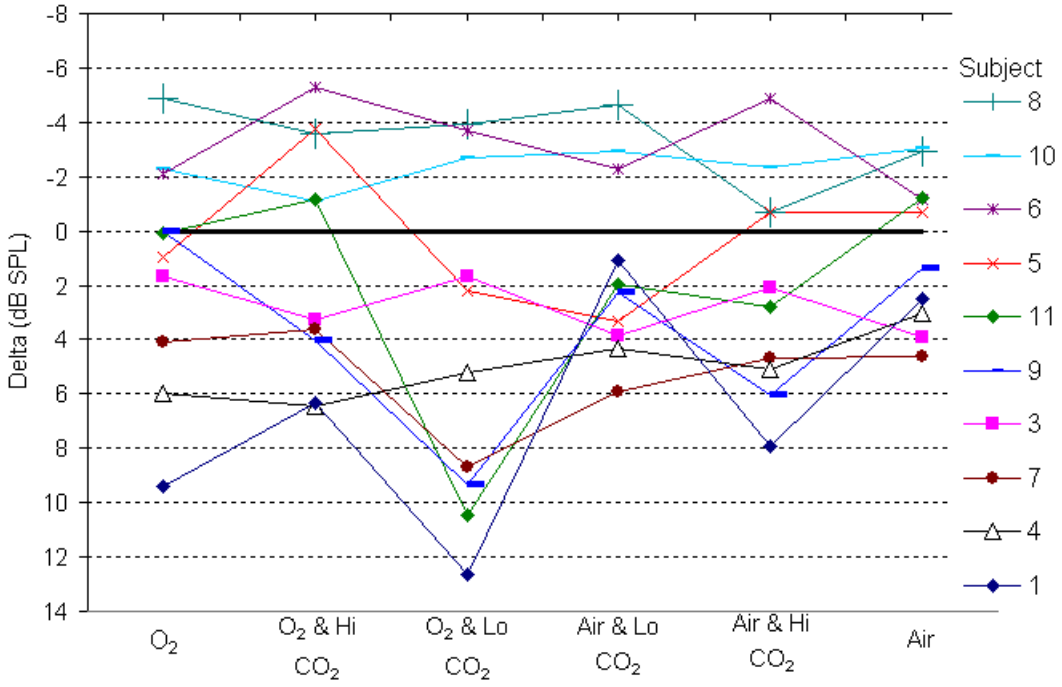

Figure 4. Individual subject SFOAE delta (baseline - gas mix) amplitudes for all gas mix conditions for 3000 Hz mean cluster. Decremental shifts are shown below 0 dB SPL.

Pairwise Comparisons of Significant Shifts

Table 4 shows if the proportional differences of decrements and increments (based on the X^2 results shown in Table 3) between selected experimental test sessions of interest are significant based on the z-test for dependent proportions. Differences in proportions are calculated as the first listed condition's proportion minus the second condition's proportion. For example, the significant difference in proportion of -.19 shows that the O_2 and low CO_2 condition's proportion of significant single frequency decrements is significantly more (.19 more) than the experimental air and low CO_2 condition. Therefore, a hyperoxic effect was found. Table 4 also shows an additional hyperoxic effect when the air and O_2 conditions are compared, as well as hypercapnic, and hyperoxic and hypercapnic combined effects. When incremental proportions were examined for single frequencies, a combined hyperoxic and hypercapnic effect was found.

Table 4. Pairwise Comparisons of Selected Decremental and Incremental Proportions of Significant Shifts for Single Frequencies

	n	Difference	95% CI	P
Decremental Effects of hyperoxia				
Air vs. O_2	97	-.16	(-.27, -.06)	.00**
Air and low CO_2 vs. O_2 and low CO_2	99	-.19	(-.29, -.09)	.00***
Air and high CO_2 vs. O_2 and high CO_2	93	.03	(-.01, .08)	.18
Decremental Effects of hypercapnia				
Air vs. air and low CO_2	99	-.08	(-.15, -.01)	.02*
Air vs. air and high CO_2	99	-.16	(-.25, -.07)	.00***
O_2 vs. O_2 and low CO_2	97	-.08	(-.19, .02)	.13
O_2 vs. O_2 and high CO_2	95	.04	(-.04, .12)	.29
Decremental Effects of hyperoxia and hypercapnia				
Air vs. O_2 and low CO_2	98	-.28	(-.39, -.16)	.00***
Air vs. O_2 and high CO_2	97	-.14	(-.23, -.05)	.00***
Incremental Effects of hyperoxia and hypercapnia				
Air vs. O_2 and high CO_2	96	-.17	(-.25, -.09)	.00***

$^{*}p < .05$. $^{**}p < .01$. $^{***}p < .001$.

Group Delay Individual Analyses

In Table 5 a decrement (unwrapped phase slope is shallower) in group delay represents a wider cochlear filter bandwidth. An increase in group delay (unwrapped phase slope is steeper) means the filter bandwidth is narrower. Because 5% of shifts are expected to be beyond 2 *SD*, all expected counts for $n = 19$ and $n = 20$ are = 1. Using the chi-square statistic with Yates' correction applied due to the small size of expected counts equal to 1, no gas mix condition

showed a significant proportion of shifts in either direction. Since the proportion of shifts for individuals was not significant, no further analysis of the group delay measure was pursued.

Table 5. Significant Group Delay Shifts from Baseline

	Decrement		Increment	
Gas-Mix		X^2_{adj}		X^2_{adj}
Air	5%	0.00	0%	0.26
Air and low CO_2	10%	0.26	5%	0.00
Air and high CO_2	0%	0.26	5%	0.00
O_2	11%	0.26	0%	0.26
O_2 and low CO_2	0%	0.26	10%	0.26
O_2 and high CO_2	10%	0.26	5%	0.00

Note. X^2_{adj} with Yates' correction applied. Degrees of freedom = 1. Some data are missing due to data criteria restrictions. Includes all clusters.

Mean Group SFOAE Amplitudes

For the 3000 Hz mean cluster amplitudes, when averaged across the group, Figure 5 shows baseline SFOAEs exceeded all other measured conditions, including the identical experimental air condition that showed the second highest mean amplitudes. To determine if these mean amplitudes for any of the experimental gas conditions (including the air condition, but excluding the 100% O_2 condition due to a missing subject) were significantly different from the mean SFOAE amplitudes measured at baseline, a one-way repeated measures analysis of variance for fixed effects was done resulting in a significant difference among the gas-mixes, $F(4, 36) = 2.73$, $p < .05$). While all gas mixes did result in lower mean SFOAE amplitudes, a Dunnett's multiple comparison test showed only the O_2 & low CO_2 gas mix condition resulted in significantly ($p < .05$) lower SFOAE amplitudes than those obtained during the baseline air condition. This finding was consistent with the SES analysis that showed that the largest percentage of individual SES decrements was also found for this condition (Table 3).

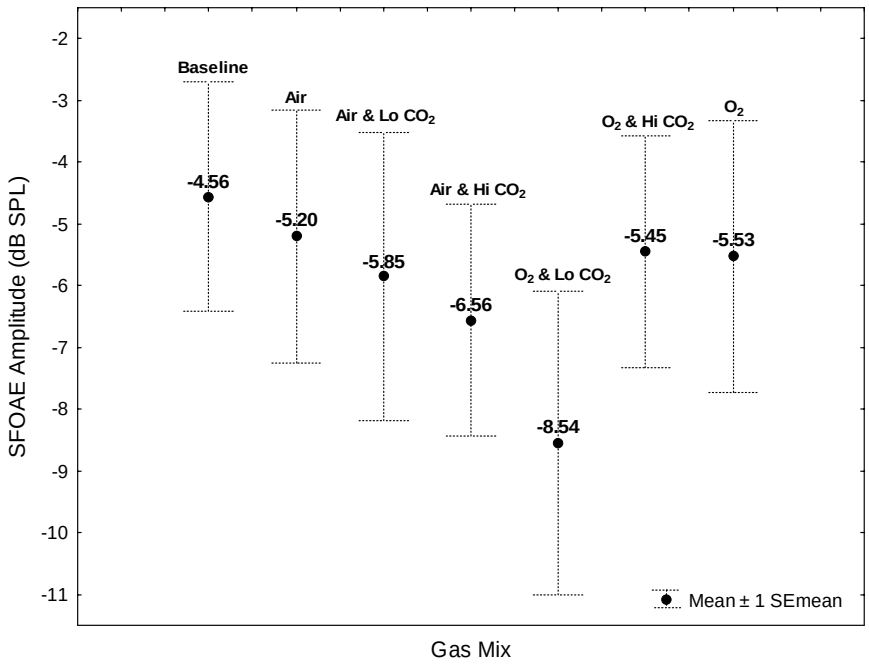

Figure 5. Mean SFOAE amplitude by gas mix for 3000 Hz mean cluster.
Figure includes 100% O_2 condition that was omitted from the ANOVA due to missing data for participant 9. Only the O_2 & Low CO_2 condition was found to be significantly different in amplitude from the baseline.

Changes in End Tidal Carbon Dioxide Levels

As expected, as inspired levels of carbon dioxide increased the end tidal carbon dioxide levels also increased. Figure 6 shows that when breathing the control gas condition (room air), the resulting $F_{ET}CO_2$ shows large variability (ranging from 3-7%). However, as the inspired CO_2 levels increased, variability in $F_{ET}CO_2$ is shown to decrease. Breathing the highest levels of carbon dioxide (6%) resulted in $F_{ET}CO_2$ ranging from about 6-8%.

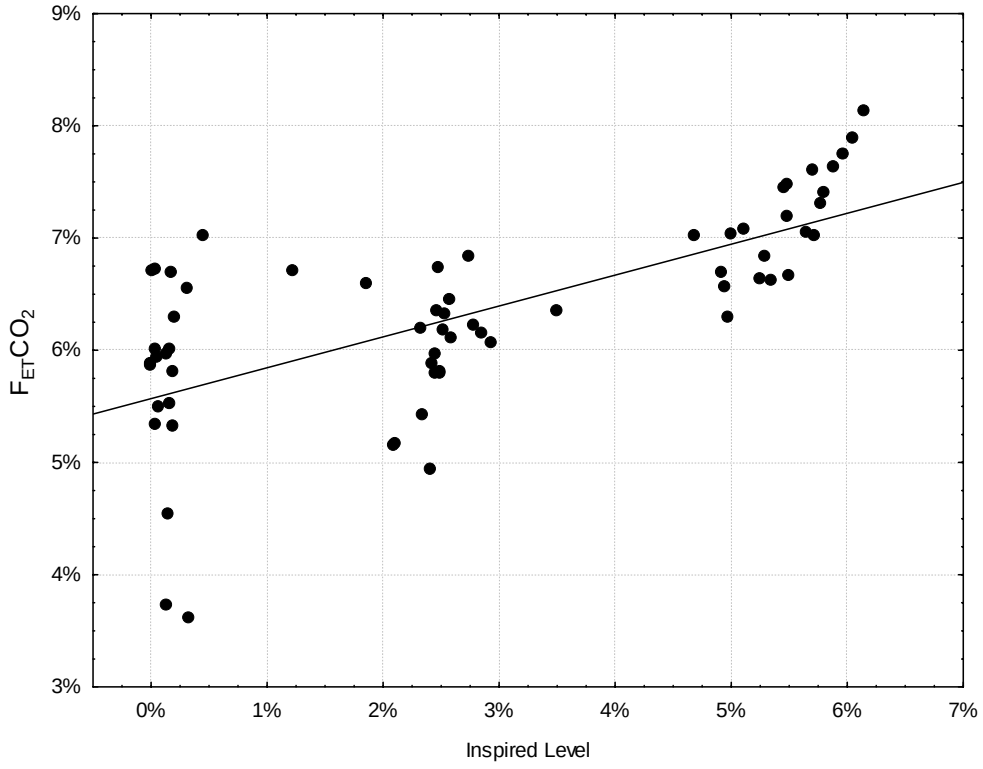

Figure 6. Relationship between $F_{ET}CO_2$ and inspired level CO_2 levels for both air and experimental gas conditions for 8 participants.

Relationship between SFOAE Mean Cluster Amplitude and End Tidal Carbon Dioxide Levels

The scatterplot of SFOAE amplitudes for the 3000 Hz cluster frequencies as a function of $F_{ET}CO_2$, with data points labeled by subject is displayed in Figure 7. These data show that for individuals with lower SFOAE amplitudes, increasing SFOAE amplitudes appear to be associated with increasing CO_2 level, but for individuals with higher SFOAE amplitudes, decreasing SFOAE amplitudes appear to be associated with increasing $F_{ET}CO_2$. To determine if this relationship was statistically significant a piecewise regression was done. With SFOAE amplitude (dB SPL) as the dependent variable, the initial predictor variables were $F_{ET}CO_2$ (%), inspired CO_2 level (%), and breathing frequency rate. Using Statistica (StatSoft, 2005), the best change point (knot) for SFOAEs was selected at – 6.51 dB SPL. Although the overall model's results were robust, *adjusted* $R^2 = .80$, $F(7, 57) = 38.71$, $p < .001$, an examination of each predictor's contribution to the model showed that only $F_{ET}CO_2$was a significant predictor of SFOAE amplitude ($p = .01$). Since neither inspired CO_2 levels nor breathing rates contributed significantly to the prediction of SFOAE amplitude, a final piecewise equation was done excluding these predictors and including only $F_{ET}CO_2$. This final model shows that $F_{ET}CO_2$ accounts for a significant proportion of variance in SFOAE amplitudes ($R^2 = .81$, $F(3, 61) = 91.56$, $p < .001$). These results reduce to the following two models for low and high SFOAEs, respectively:

Low SFOAEs ≤ -6.51:
SFOAE = -15.66 + 76.97 * $F_{ET}CO_2$
High SFOAEs > -6.51:
SFOAE = -9.47 –157.57 * $F_{ET}CO_2$

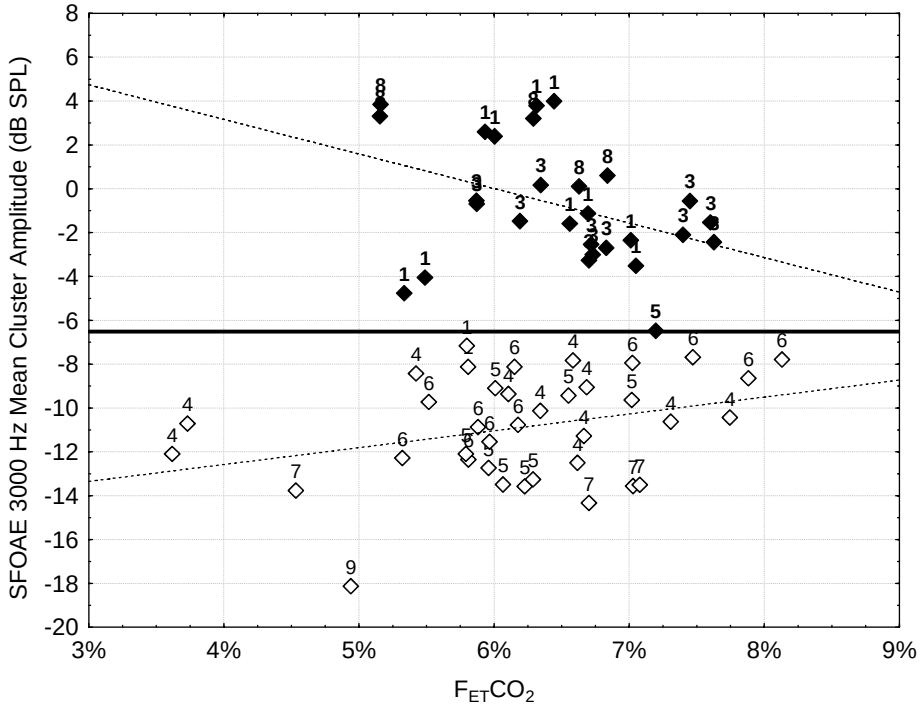

Figure 7. SFOAE 3000 Hz mean cluster amplitude (dB SPL) as function of $F_{ET}CO_2$ levels for the low and high SFOAE groups.

DISCUSSION

The objectives of this study were to determine if elevated levels of oxygen and/or carbon dioxide in the cochlea would result in a significant change in cochlear processing as reflected in SFOAE amplitudes and/or frequency tuning capabilities in human subjects across a portion of the speech frequency range.

The stimulus frequency otoacoustic emission test (Shera & Guinan, 2002; 1999; Guinan, 1990; Kemp et al., 1990) was used to assess cochlear sensitivity (SFOAE amplitude) and tuning (SFOAE group phase delay) in a normal hearing, human group that consisted of six divers and four non-divers. A significant proportion of decremental and incremental shifts were found for single frequency and mean cluster SFOAE amplitudes. Overall, 29% of the individual frequency SFOAE tests and 33% of the cluster SFOAE amplitudes exhibited an SES and there were three times as many significant decreases as increases in single frequency SFOAE amplitude. High oxygen levels in the cochlea, when produced by hyperoxia alone or in combination with hypercapnia, appeared to have an effect on cochlear processing after only five minutes of breathing a particular hyperoxic, hypercapnic, or combination of a hyperoxic and hypercapnic gas mix. The results also suggest that SFOAE amplitude is related to the $F_{ET}CO_2$ of the individual. In other words, the effect on cochlear processing appeared related to the effects of the breathing gas. The greatest effects were seen for the 97% O_2/2.5% CO_2 gas mix, which was a surprising finding since a significant effect was not observed for exposure to the 94% O_2/6% CO_2 gas mix condition that should have resulted in high levels of oxygen saturation in the inner ear. The amount of O_2 delivered to the cochlea will depend on the combined effects of O_2 saturation level and blood flow to the cochlea. The concentration of oxygen in the breathing mix will affect the O_2 saturation level, while blood flow volume will be affected by the level of CO_2 retained in the blood. Volume of blood flow in the cochlear artery and carbon dioxide levels in the blood were not measured, therefore, we do not know which gas condition produced the greatest level of oxygen within the cochlea. However, it was anticipated that the highest O_2 saturation levels in bodily tissues occurred with the combination hyperoxic/hypercapnic gas mixes. Interpretation of the findings is also limited given the number of individuals tested and the limited data points used for the $F_{ET}CO_2$ fractions.

Limitations of this study were that only 10 individuals were tested, not enough tests were performed to adequately assess the recovery function for the experimental gas mixes, and not all of the breathing rate, inspired levels, and end tidal data were saved. However, strengths of this study were that we obtained a substantial amount of normative data during session two on normal hearing subjects that were consistent with Lapsley Miller et al. (2004) results, showing that the SFOAE test appears to be as reliable as the TEOAE and DPOAE procedures.

The results are interesting in that significant emission shifts (SESs) occurred in both positive and negative directions. This was an unexpected finding that may pose more questions than answers. On the one hand, the clear majority of SESs that were decrements in SFOAE amplitude could help support the theory that high levels of ROS within the cochlea has a detrimental effect on the peripheral auditory system (Kopke et al., 1999; 2001). It was also interesting that data for seven individuals fell out into “low” and “high” SFOAE amplitude groups and the subjects in the low

group tended to show a significant increase in amplitude while those in the high group tended to show significant decreases in amplitude. These findings, of course, need to be interpreted with extreme caution as there were a small number of people tested and these differences could average out with a larger number of participants. A possible explanation could be that while there may be a true negative relationship between the higher robust SFOAEs and end tidal carbon dioxide levels, the weaker SFOAEs may not be related to $F_{ET}CO_2$ in any measurable sense. This is an area that requires additional study prior to making any conclusions.

The findings of this study also indicate that elevated levels of oxygen and carbon dioxide did not appear to have an effect on SFOAE phase group delay or auditory tuning. At this time, with this limited number of individuals, it is not known whether this is because auditory tuning as measured by the SFOAE is more robust than amplitude (and therefore less susceptible to change), or if the group-delay measure is not sensitive enough to measure sub-clinical changes in cochlear functioning secondary to hyperoxia and hypercapnia. It is also possible that there is no effect.

CONCLUSIONS

As documented by SFOAE testing, hyperoxia and hypercapnia, both individually and in combination, did appear to have effects on cochlear processing. Whether or not these findings are related to harmful or protective effects in the cochlea is not known and further study in this area with a larger number of subjects is recommended as Navy Special Forces divers and saturation divers do appear to be at risk for hearing loss (Cudahy & Avila, 1998). These are special populations within the U.S. Navy diving community and identifying their risk for auditory harm from hyperoxia/hypercapnia in addition to noise-exposure could identify them as candidates for new types of antioxidant therapy currently being tested (Kopke et al., 2001). Further research in this area in a larger number of subjects would also increase our understanding of the effects of hyperoxia and/or hypercapnia physiological stressors on the peripheral auditory system.

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