UNIVERSITY OF SOUTHAMPTON

The Division of Clinical Neurosciences

School of Medicine

And

The Medical Research Council Institute of Hearing Research Southampton

PROPERTIES OF MAXIMUM LENGTH SEQUENCE AND NONLINEAR VOLTERRA SLICE OTOACOUSTIC EMISSIONS

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Thesis for the degree of

Doctor of Medicine

June 2008

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

THE MEDICAL RESEARCH COUNCIL INSTITUTE OF HEARING RESEARCH SOUTHAMPTON AND THE DIVISION OF CLINICAL NEUROSCIENCES SCHOOL OF MEDICINE

Doctor of Medicine

PROPERTIES OF MAXIMUM LENGTH SEQUENCE AND NONLINEAR VOLTERRA SLICE OTOACOUSTIC EMISSIONS

by Hasnaa Ismail-Koch

Evoked otoacoustic emissions (EOAEs) are produced by the cochlea and provide an objective and non-invasive measure of cochlear function. A new technique, based on Maximum Length Sequences (MLSs) enables stimulus rates of up to 5000 clicks/s to be used, and gives increased speed and sensitivity of testing. Volterra slice otoacoustic emissions (VSOAEs) can be extracted from the response using this technique. These represent nonlinear temporal interaction components and are more sensitive to changes in cochlear pathology than the conventional response. Conventional EOAE amplitude differs between ears and sexes; female subjects having responses of greater amplitude than male subjects and right ears larger responses than left ears. As a pre-requisite to clinical use it is necessary to establish if these differences occur with the Maximum length sequence otoacoustic (MLSOAE) technique and with VSOAEs and whether they change with stimulus rate, order or slice. The relationship between VSOAEs, Spontaneous otoacoustic emissions (SOAEs), Distortion product otoacoustic emissions (DPOAEs) and the input/output function (I/O) for click-evoked OAEs (CEOAEs) recorded at the conventional rate (40 clicks/s) was also investigated to assess if these measures of cochlear nonlinearity were related to one another.

In the first set of experiments 80 ears of normally hearing adults were tested. MLSOAEs were recorded at eight stimulus rates and two stimulus levels. For the second and third experiments 45 ears of normally hearing adults were tested. SOAEs, DPOAEs, the input/output function (I/O) for CEOAEs at the conventional rate (40 clicks/s) and at four stimulus levels, and VSOAEs at three stimulus rates were recorded.

Female subjects were found to have statistically significantly larger MLSOAEs than male subjects and gave larger amplitude responses in their right ears. This sex difference was observed with VSOAEs. A rate effect was also demonstrated with the amplitude of the MLSOAEs decreasing with an increase in rate. The VSOAE amplitude was greater for the second order compared with the third order response, and slice one had a greater amplitude than slice two. VSOAEs of higher amplitude were obtained in SOAE-positive ears. There was a significant relationship between the slope of the I/O function of the CEOAE and the VSOAEs.

The study has provided normative data for MLSOAE testing and for VSOAEs. The data obtained suggest that the amplitude (CEOAE I/O function) and temporal (VSOAEs) nonlinearities arise from the same generators, whereas the frequency domain nonlinearities (SOAEs & DPOAEs) have different generators. MLSOAEs and VSOAEs have great potential for clinical use.

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DECLARATION OF AUTHORSHIP

I, **Hasnaa Ismail-Koch**, declare that the thesis entitled

Properties of Maximum Length Sequence and Nonlinear Volterra Slice Otoacoustic Emissions

and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- This work was done wholly or mainly while in candidature for a research degree at this University;
- Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
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- Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- Part of this work has been published as:

Ismail, H. and A.R. Thornton, *The interaction between ear and sex differences and stimulus rate.* Hear Res, 2003. **179** (1-2): p. 97-103.

Signed:

Date: 24th June 2008

ACKNOWLEDGEMENTS

This project was made possible by Professor Roger Thornton of the Medical Research Council Institute of Hearing Research, Southampton and Mr John Carruth, retired Ear, Nose and Throat Consultant. I am greatly indebted to my two supervisors Professor Roger Thornton and Dr Ben Lineton, for their valuable support and patience throughout this period of research. Their contributions have been invaluable from the outset and have been fundamental to the success of this thesis. For this, and for helping me to progress in my chosen career path as an Ear, Nose and Throat surgeon, I would like to express extreme gratitude to them. They have been outstanding teachers, provided inspirational guidance and warm friendship.

My limited laboratory experience before commencing at The Institute of Hearing Research meant I would not have progressed very far without the guidance and support of colleagues there. I would like to express extreme gratitude to Jessica de Boer who taught me the basics of maximum length sequences and volterra slice otoacoustic emissions and helped me enormously in early experiments and when analysing my data. Her contagious smiles, continual encouragement, advice and friendship saw me through difficult times when experiments were not going as planned.

Sue Mooney and Dave Olleys' support and encouragement merit special attention. I must also praise the generosity of the Medical Research Council who provided the equipment necessary for these experiments.

I would like to thank all those that made this project so enjoyable and for all those 'little things' that made such a huge difference: Jemma Hine, Brigitte Lavoie, Gerry Madden, Mike Pringle, Saliya Caldera, Roberto Puxeddu, Anne Davis, Hugh Cox, Gareth John, Simon Dennis, Rami Salib, Melanie Collins, Phil Harries, Raj Mehta, Nigel Bleach, Chris Aldren, Steve Wood, Tony Jefferis, Angus Waddell, Sue Chalstrey, Deepak Gupta, Patrick Donnelly, Graham Banfield and Chris Randall!

Finally, I would like to acknowledge the immense encouragement of my family who have always been there for me, despite my neglect of them. I have greatly missed my husband Matthew, who is my life, and my children Virginia and Lyla, who have taught me how to thoroughly enjoy life. In particular I would like to thank my mother Sanaa, a truly exceptional being, who has always been incredibly hardworking, supportive and so humble, despite adversities, and who every day inspires me to be the best I can.

ABBREVIATIONS

AABR	Automated auditory brainstem response
ABR	Auditory brainstem response
BF	Best frequency
BM	Basilar membrane
CAM	Cochlear active mechanism
CEOAE	Click-evoked OAE
CF	Characteristic frequency
CM	Cochlear microphonic
DP	Distortion product
DP gram	DPOAE gram (graph)
DPOAE	Distortion product OAE
EOAE	Evoked otoacoustic emissions
IHC	Inner hair cells
IHR	Institute of Hearing Research
I/O	Input-output
NHS	National Health Service
NHSP	Newborn hearing screening programme
NIHL	Noise induced hearing loss
NLTIC	Nonlinear temporal interaction components
MLS	Maximum length sequence
MLSOAE	Maximum length sequence OAE
MRC	Medical Research Council
OAE	Otoacoustic emissions
OHC	Outer hair cells
SNR	Signal-to-noise ratio
SOAE	Spontaneous OAE
STCs	Supression tuning curves
TEOAE	Transient-evoked OAE
UNHS	Universal newborn hearing screening
VK	Volterra kernel
VS=VSOAE	Volterra slice OAE

CHAPTER 1
INTRODUCTION

1.1) The ear

1.1.1) Cochlear anatomy and physiology

The ear is the sensory organ responsible for hearing and is composed of three parts termed the outer/ external ear, middle ear and inner ear (**Figure 1.0**).

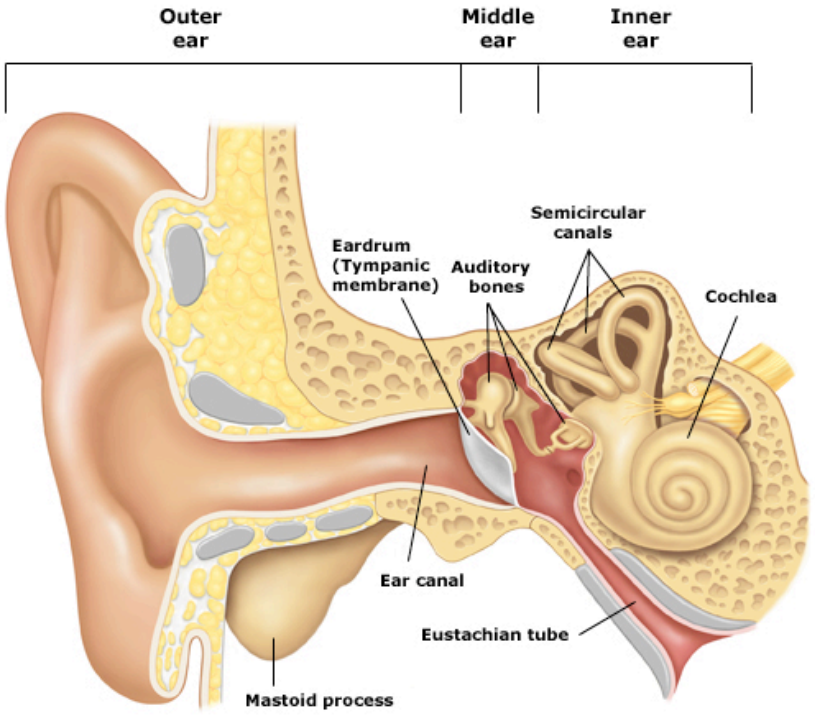


Figure 1.0. *The Ear.*

The external ear includes the auricle (pinna) and external auditory canal. The external auditory canal extends from the conchal cartilage of the auricle to the tympanic membrane, and is approximately 25 mm long in the adult. It courses slightly anteriorly and inferiorly in the adult. The middle ear is composed of the tympanic membrane, the tympanic cavity, the ossicles and the eustachian tube. The tympanic membrane forms the lateral wall of the middle ear. The inner ear consists of two main parts, the cochlea (end organ for hearing) and the vestibule and semicircular canals (end organ for balance).[1]

The cochlea resembles a snail and can be thought of as a canal that spirals around itself similarly to a snail. It makes roughly 2 ½ to 2 ¾ turns. The bony canal of the cochlea is divided into an upper chamber, the scala vestibuli and a lower chamber, the scala tympani by the membranous (otic) labyrinth also known as the cochlear duct (see **Figures 1.1**& **1.2**).

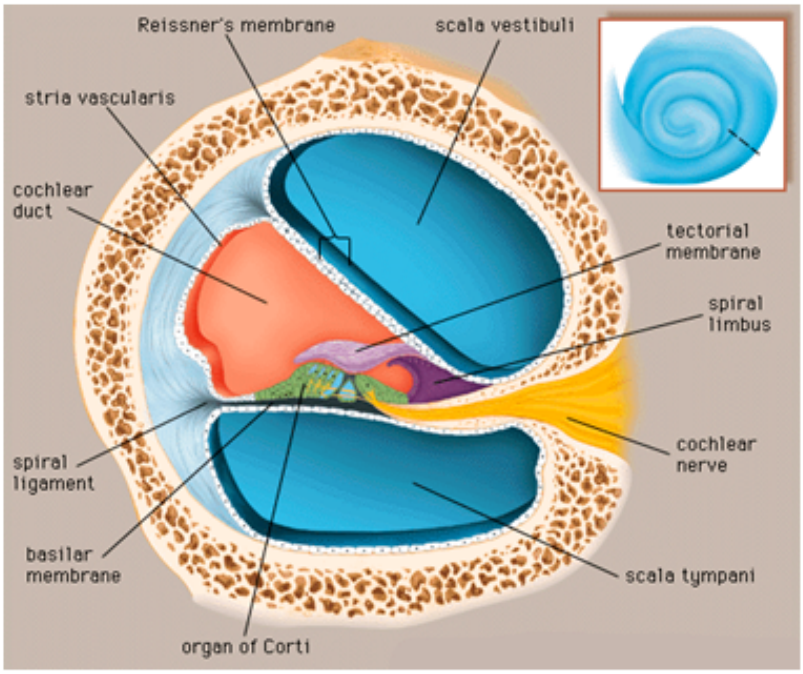


Figure 1.1. *Cross Section of the Cochlea.*

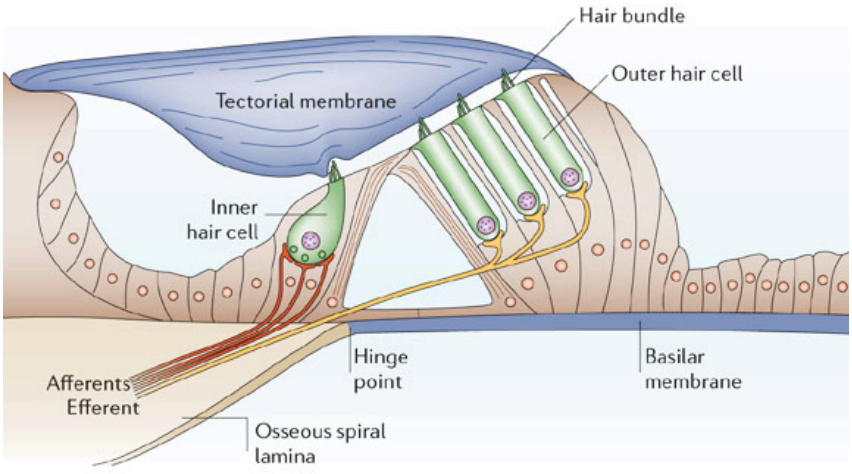


Figure 1.2. *The Organ of Corti.*

The scala vestibuli and scala tympani contain perilymph which resembles extracellular fluid and is low in potassium and high in sodium. The scala media contains endolymph which has a similar ionic content to intracellular fluid, high Potassium, low Sodium. The cochlear duct contains several different types of specialized cells responsible for auditory perception. The basilar membrane (BM) forms the floor of the scala media and the roof is formed by Reissner's membrane. Situated on the basilar membrane is a single row of inner hair cells (IHCs) medially and three rows of outer hair cells (OHCs) laterally. The cells have specialized stereocilia on their apical surfaces. A fibrous structure called the tectorial membrane attaches to the medial aspect of the scala media. It lies above the inner and outer hair cells coming in contact with their stereocilia. The base of the hair cells synapse with dendrites from the auditory nerve. The auditory nerve leaves the cochlear and temporal bone via the internal auditory canal and travels to the brainstem.[1]

For physiological purposes, the ear is divided into two parts - the conducting apparatus, consisting of the external ear, tympanic membrane, chain of ossicles, eustachian tube and labyrinthine fluids; and perceiving (sensorineural) apparatus, consisting of the end-organ (organ of Corti, **Figure 1.2**), auditory division of VIIIth cranial nerve, and central connections.[2] The transmission of sound to the inner ear most commonly occurs by way of the ossicular chain, from the vibrating tympanic membrane to the oval window (**Figure 1.3**). The conduction of sound may also occur directly across the middle ear when waves fall on the round window, for example if there is a large perforation of the drumhead, or by bone conduction where sound is taken up and transmitted to the inner ear through the bones of the skull [2]

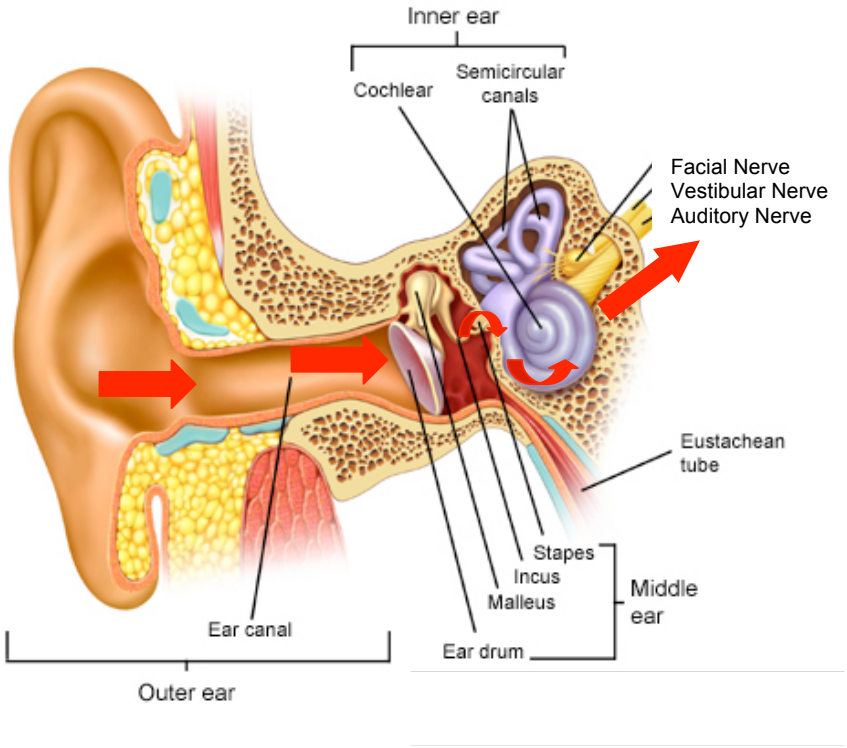


Figure 1.3. The transmission of sound to the inner ear.

When the sound signal impinges on the oval window, the cochlea transforms the signal from mechanical energy into hydraulic energy and then, at the hair cells, into electrical energy.[3] The fluids within the cochlea are incompressible; hence, pressure anywhere along the cochlea is instantly transferred to other points. As the footplate of the stapes moves in and out of the oval window, a travelling wave is created in the cochlea.[3] As the wave travels through the cochlea, it causes movement of the basilar membrane, which results in a 'shearing motion of the cilia of the inner and outer hair cells. This motion depolarises the inner and outer hair cells, and produces the cochlear microphonic (CM). The CM is thought to be due to both the IHCs and OHCs, but probably more from the OHCs, and is probably the final mechanical event preceding neuronal stimulation.[2, 3]

The cochlea functions as a transducer and analyser of input frequency and intensity. The cochlea is organised spatially according to frequency (tonotopic). The place theory proposes that for every frequency there is a highly specific place (called the characteristic place) on the basilar membrane where the hair cells are maximally

sensitive to that frequency, the basal end for the higher frequencies and the apical end for low frequencies. However, this cannot explain the extraordinary frequency resolving properties of the auditory system. [3]

Bekesy's travelling wave theory is that the disturbance of the cochlear fluids causes an energy wave to travel from base to apex along the basilar membrane until the wave reaches a maximum.[3]The point of maximum displacement is determined by the interaction of the frequency of the sound and the stiffness and mass of the basilar membrane; this also does not account for such sharp frequency analysis.[3]

The OHCs are electromotile reacting mechanically to the incoming signal by shortening and lengthening according to their characteristic (best) frequency (CF/BF).[3] The consequence of this motility is to amplify the motion of the basilar membrane at the specific location (frequency) of the OHC making the IHCs in the same region 30 to 40 dB more sensitive.[4] Under strong efferent impulse, the OHCs are part of an active feedback mechanism, adjusting the physical properties of the basilar membrane so that a given frequency maximally stimulates a narrow group of IHCs.[3] For multiple frequencies (complex sound), travelling wave maxima occur at several points, and the cochlear apparatus constantly tunes itself for best reception and encoding of each component frequency.[3]

The cochlea is nonlinear, acting like a compression circuit by reducing a large input range into a much smaller output range.[3] The compression mainly occurs around the OHCs characteristic frequency. This nonlinearity allows the auditory system to manage a very wide range of intensities, which is represented by the nonlinear logarithmic decibel scale.[3]

1.1.2) The cochlear amplifier

The human auditory system has a remarkable ability to discriminate sounds that differ by no more than a few cycles per second or by a few decibels. George von Bekesy, as mentioned before, viewed the role of the human cochlea as purely passive and suggested that tuning of the response that is necessary to achieve pitch and intensity discrimination occurs in the central portion of the auditory pathway.

Following the Second World War in 1948, Thomas Gold, an astrophysicist who had worked on radar during the war, suggested it was possible to include a positive feedback in receiver design to improve sensitivity; applied to the cochlea frequency selectivity of the cochlea could be enhanced if a source of mechanical energy were present within the cochlea, thus the cochlea is an active participant in tuning the auditory response.[5] He speculated that the cochlea has a positive feedback system that would produce spontaneous emissions.[5]

This theory was largely ignored until the discovery of ‘echos’ being emitted with a short delay by the ear when stimulated with brief acoustic stimuli later termed as otoacoustic emissions by Kemp in 1978.[6] The cochlear amplifier is essentially a positive feedback loop within the cochlea that amplifies the travelling wave. Vibrations within the organ of Corti are sensed and increased in magnitude by forces in synchrony. The increased vibrations result from outer hair cell motility and stereociliary active bundle movements.[7] Outer hair cells are assumed to feed cycle by cycle force (electromotile response) to the basilar membrane so that its vibration is amplified at the best frequency.[8] These processes may be regulated by the intracellular ionic composition, the lipid components of the outer hair cell plasma, and the structure of the outer hair cell cytoskeleton. [7]

1.2) Otoacoustic Emissions (OAEs)

1.2.1) Origins of OAEs

Otoacoustic emissions (OAEs) are sounds that are produced by healthy ears in response to acoustic stimulation. They are considered to be epiphenomena, and by-products of the activity of the outer hair cells in the cochlea occurring as a by-product of a unique and vulnerable cochlear mechanism known as the 'cochlear amplifier' described above.[5, 6] These emissions are recorded in the ear canal when the tympanum receives vibrations transmitted through the middle ear from the cochlea.[6] OAEs are thought to arise by at least two fundamentally different mechanisms within the cochlea: nonlinear distortion and linear reflection.[9]

Otoacoustic emissions were first described by Kemp in 1978. His discovery was initially greeted with skepticism, but OAEs have since been reliably confirmed.[5]

The cochlear amplifier is physically essential to the high sensitivity of hearing and to the formation of a sharp 0.25 octave tonotopic "image" of the acoustic environment along the length of the cochlea. Basilar membrane disturbances that escape from the cochlear amplifier mechanism and travel away from the sensory cells back to the base of the cochlea result in the occurrence of OAEs in the ear canal. In the cochlea the vertical motion of the BM exerts a differential oscillating fluid pressure on the oval and round windows causing the ossicles and subsequently the ear drum to vibrate thereby producing OAEs in the ear canal.[6]

The cochlear amplifier mechanism must be present and to some degree operational in order for OAEs to exist. Paradoxically, the reasons why vibrations are sent back to the base to form OAEs all relate to natural imperfections in this mechanism.[6] When OHC motility is not completely uniformly distributed, a stimulus frequency OAE will be generated. Not only spatial imperfections can generate OAEs, if the forces exerted by OHCs on the BM do not exactly follow the stimulus waveform (for example

if the OHC motility is nonlinear), they will add distortion products to the forward travelling wave. Under conditions of high amplification, endless recirculation of the travelling wave leads to sustained oscillation inside the cochlea and to spontaneous OAE of one or more pure tones into the ear canal.[6]

Different locations within the cochlea may contribute to a single frequency of an OAE and these may affect each other. As will be described later the transmission back to the ear canal also depends on the individual middle ear characteristics. The interplay of all these factors cannot yet be accurately modelled, not least because most parameters are unknown. This accounts partially for the great variation between individual healthy ears in the level and the spectrum of the OAEs they exhibit. Stimuli of different frequencies or spectral composition can give rise to variable OAE waveform patterns, and therefore a more meaningful description of cochlear status is to take an average OAE characteristic over a range of stimuli.[6]

OAEs usually arise only in frequency bands where hearing is near normal, as they are frequency specific responses. Thus they are a useful indicator to normally and abnormally functioning parts of the cochlea. Changes in cochlear status can thus be detected by OAEs which show a high sensitivity to any change in cochlear status. Overall, OAE responses may carry a large amount of information about the status, activity and environment of OHCs, which we are at present unable to interpret. OAEs provide the only detailed non-invasive window on the cochlea and by their very presence confirm normal presynaptic cochlear function.[6]

Nonpathologic problems may result in the absence of OAEs. These include a poor seal or incorrect probe tip placement (the equipment will usually indicate there is a problem). Standing waves may also occur but the equipment will usually alert the clinician of the error. Debris, cerumen, foreign bodies, or vernix caseosa in the case of neonates may also result in the absence of OAEs.[10] In the first three days of life the pass rate for OAEs changes. There is an increasing pass rate of about 40% on day 1 to a pass rate of about 70% on day 3 in the absence of any middle-ear or

external ear problems.[10] To account for the poor emission on day 1 and improvement in the emission waveform thereafter, it has been suggested that changes in the emission in the post-partum period are due to oxygenation of the cochlea. This is thought to occur as in utero there is a reduced oxygen level in the cochlea due to a decreased oxygen saturation level. At birth with the commencement of breathing the neonatal oxygen tension rises, however the outer hair cells require a critical period of time with normal oxygen tension before they become fully functional.[10] Thus if OAEs are obtained during this period, as is common with the newborn hearing screening programme and early hospital discharge they are treated with caution if there is a fail and repeated later in the community or in hospital. In the case of premature births or neonates with complications Auditory brainstem responses (ABRs) are also recorded.

The patient needs to be still and quiet, hence in the uncooperative patient recordings cannot be obtained.

Pathologic problems that can adversely affect OAEs include outer ear stenosis, otitis externa and ear canal cysts. In the middle ear abnormal ear pressures and glue ear may result in absent OAEs. Perforations of the tympanic membranes and ventilation tubes do not necessarily prevent good recordings.[11] When testing for transiently evoked otoacoustic emissions (TEOAEs) immediately after grommet insertion in theatre using the ILO88 (commonly used in the newborn hearing screening programme), research has shown that TEOAEs can be recorded in 50% of ears immediately after grommet insertion, but the responses are reduced compared with normal ears; hence, it is not an accurate screening technique.[12] In the case of distortion product otoacoustic emissions (DPOAEs) it has been shown that effusion in the middle ear reduces the number of measurable responses and their amplitude in the whole range of frequencies from 0.5 to 8 kHz.[13] These changes were more distinct in mucous than in serous effusion. There was an increase in the number of measurable responses and amplitude of DPOAE after surgery. The researchers

concluded that inserted tympanostomy tubes have no influence on the feasibility of DPOAE recording but reduced their amplitude in comparison to the control group.[13]

Middle ear conditions such as otosclerosis, middle ear disarticulation, cholesteatoma , cyst and bilateral (or unilateral) otitis media may also result in an altered OAE response. In the case of otis media with effusion, in order to obtain OAEs, the cochlear response must be able to travel efficiently through the middle ear and tympanic membrane to the recording microphone placed in the ear canal. In the presence of normal cochlear function, OAEs generally are absent in the presence of middle ear effusions. Therefore, it is best to conduct OAE testing after the effusion has resolved. If otoacoustic emission testing is required prior to the resolution of middle ear effusions attempts to record OAEs are not contraindicated. The presence of an OAE as may occur in otitis media with effusion is a useful indicator, the absence allows no clear conclusion of cochlear function to be made.[11]

Within the cochlea itself any cochlear pathology may affect OAEs. In addition exposure to ototoxic medication or to noise may affect OAEs and changes in the OAE may become apparent prior to any change in the behavioural audiogram. Vestibulocochlear nerve conditions may also affect OAEs; for example if a vestibular schwannoma results in the decrease of the blood supply to the cochlea then the OAE is altered.[11]

Certain conditions may elicit abnormal OAEs with normal behavioral thresholds. These conditions include tinnitus in which OAEs may be abnormal in the frequency locus of the tinnitus.[11] Noise exposure as described above may result in an altered OAE with normal audiogram appearances. Ototoxicity and vestibular pathologies may also result in abnormal OAEs with a normal audiogram.[11]

Alternatively some conditions result in the production of normal OAEs and abnormal behavioral thresholds. Non organic hearing loss, attention deficits, autism and possibly, inner hair cell damage but normal outer hair cells (reported for animals but

no human reports yet) may all elicit normal OAEs but abnormal behavioural audiograms thus providing a good indicator of cochlear function, in particular outer hair cell health.[11]

Auditory neuropathy is a condition which may affect the inner ear to the brain; this includes central auditory nervous system dysfunction and CN VIII auditory dysfunction. In this condition, the OAE may be normal but the ABR is abnormal.

OAEs are subdivided according to the type of stimulus that elicits them. Two distinct classes can be identified.[14] The first major emission type is referred to as a spontaneous otoacoustic emission (SOAE):, these emissions are spontaneously present without external acoustic stimulation.[14] In the other principal class the emissions are evoked by different kinds of acoustic stimulation, and are further divided into three subclasses according to the type of the eliciting stimulation.[14] The subclasses are transiently evoked OAEs (TEOAEs), stimulus-frequency (SFOAEs) and distortion-product OAEs (DPOAEs).

1.2.2) Types of otoacoustic emissions: SOAEs, TEOAEs, SFOAEs, DPOAEs

1.2.2.1) Spontaneous Otoacoustic Emissions (SOAEs)

These consist of narrow-band signals that can be measured in the absence of deliberate acoustic stimulation.[14] Spontaneous emissions are stationary signals that can be recorded over long periods of time, both within and between experimental sessions.[14] They are measured using a securely fitting probe in the ear canal.

‘Objective tinnitus’ which has been described in the literature by Loebell (1962) probably represents a form of SOAEs. [14] The spectral analysis of an SOAE was first described in 1970 by Kumpf and Hoke.[15] Kemp (1979) was the first to discover SOAEs in clinically normal ears.[16]

SOAEs were initally found in approximately one-third of the ears of normally hearing individuals.[14] In the principal studies (Zurek, 1981; Tyler and Conrad-Armes , 1982; Hammel , 1983; Schloth, 1983; Bright and Glattke, 1986; Rabinowitz and Widen, 1984; Wier et al, 1984; Dallmayr, 1985; Strickland et al, 1985; Probst et al, 1986; Lonsbury-Martin et al, 1990) over 1000 ears were examined with 34% of them exhibiting SOAEs.[14] Using subjects as the measurement unit, SOAEs were recorded in 43% of normally hearing humans.[14] In addition 13% of all ears, or 38% of the ears with SOAEs, demonstrated more than one SOAE per ear.[14] It is possible to obtain multiple SOAEs from a single ear; up to 10 or more SOAEs can be detected within a single ear (Schloth, 1983; Bright and Glattke, 1986).[14, 17] As the equipment has improved, SOAEs have been shown to be present in approximately 60% of healthy ears. In adults, the range is about 30-60% and in neonates with normal hearing, the range is approximately 25-80%.[10] SOAEs are not found in individuals with hearing thresholds worse than 30 dB HL, in most cases. In those subjects with spontaneous otoacoustic emissions, the vast majority are in the low frequency range 1000- to 2000-Hz region; amplitudes are between -5 and 15 dB SPL (usually greater than 12dB).[11, 18] Some individuals have multifrequency SOAEs over a broader frequency range.[14]

Characteristically SOAEs are bilateral rather than unilateral. When unilateral, they are more common in the right rather than in the left ear. SOAEs more often occur in females than in males (across all ages).[11, 14]

As mentioned not everyone who is of normal hearing demonstrates a spontaneous otoacoustic emission. Therefore, if SOAEs are part of the normal physiological function of the cochlea then recording techniques would be suspect. [19] The limitations of our technology should be recognised. As there is no input stimulus and therefore, no possibility of time-locked averaging, spontaneous otoacoustic emissions are recorded by frequency-delaying averaging.[19] Epochs are sampled, the Fourier transform applied and the power spectrum calculated, then this is placed into a buffer and successive recordings are averaged into the same

buffer.[19] Therefore, any spontaneous activity which is not constant in frequency cannot be represented by this recording technique.[19] Nevertheless, an amazing series of measurements have been obtained using this very simple technique.[19]

The presence of SOAEs may be influenced by external acoustic stimuli, changes in ambient temperature, or certain drugs. Aspirin ingested in doses of about 4g/day, reduced or abolished the amplitudes of SOAEs in normally hearing adults.[14]

Spontaneous otoacoustic emissions can also be detected in ears with hearing loss, although less frequently and only in frequency regions associated with normal or near-normal hearing.[20] Exceptions to these observations are the high level SOAEs from rare subjects that can be heard by others, without amplification that have been reported.[14] The patient often cannot hear these noises. These emissions are very uncommon but may coexist with sensory hearing loss. They are more common in children than in adults.[14]

The presence of SOAEs is considered a sign of cochlear health, but the absence of SOAEs is not necessarily a sign of abnormality.

1.2.2.2) Transient Evoked Otoacoustic Emissions (TEOAE's)

Transient evoked otoacoustic emissions are elicited by the use of brief acoustic stimuli; hence, the stimuli characteristics used to evoke these particular emissions are transient. **Figure 1.4** illustrates a TEOAE.

Typical characteristics of TEOAEs include nonlinear growth, with saturation at moderate levels of stimulation, frequency dispersion , and a discrete latency with respect to the stimulus onset.[14] TEOAEs are detected in normally hearing neonates, children and adults in similar proportions.[14] Kemp was the first to examine ears with sensorineural hearing losses using TEOAEs and found that emissions were absent in ears with hearing losses > 30 dB HL.[14] Noise induced

high frequency hearing loss adversely affects TEOAEs resulting in a reduction in TEOAE incidence as well as the number of dominant emission frequencies per ear.[21] TEOAEs are more sensitive to cochlear status changes than distortion product otoacoustic emissions manifested by subtle changes in the TEOAE.[6]

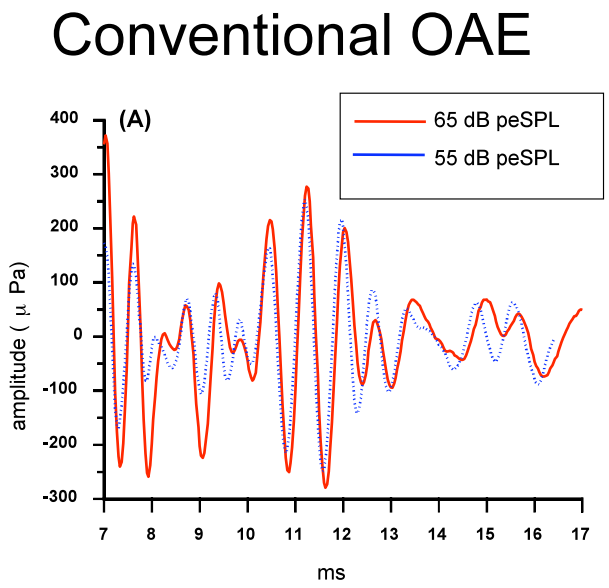


Figure 1.4. *A conventional OAE/ TEOAE.*

In a review of previous studies by Probst (1991) TEOAEs were recorded in almost all of the 1062 human ears tested in subjects with normal hearing irrespective of age or sex. This equates to TEOAEs being detected in 98% of normally hearing human ears.[14] Thus TEOAEs appear to be a general property of the human peripheral auditory system and can be recorded in most, if not all, normally hearing ears.[14]

The TEOAE duration exhibits a wide distribution ranging from several milliseconds to several hundreds of milliseconds (Wit and Risma, 1980).[14] The amplitude spectrum of a TEOAE is dependant upon several factors, including the spectral energy of the stimulus, the duration of the averaged time period, and the structurally dependant resonances unique to an individual ear.[14] On the application of a broadband stimulus and the production of a response, which is averaged over a relatively long

time period, the majority of ears have exhibited TEOAEs with spectra containing several discrete, i.e. dominant frequencies. Identical dominant emission frequencies are evoked providing the stimulus contains spectral energy at those particular frequencies, independent of stimulus type or level. Because they are related to the sound-related activation of basilar membrane mechanics, the dominant frequencies are probably produced by emission generators located at 'fixed' places along the organ of Corti.[16]

The dominant TEOAE frequencies are usually measured in the 0.5-4kHz frequency range. The remarkable stability of these frequencies is analogous to that observed for SOAEs. It is well established that the waveforms of the TEOAEs are dependant both on the number and tuning of the 'fixed' emission frequencies in addition to the spectrum of the stimulus.[22]

Emission frequencies generally add linearly, consequently the emitted response to a click may be reproduced by adding the responses to individual tone bursts placed at the dominant-emission frequencies.[23, 24] However, complicated, nonlinear interactions such as occur with SOAEs cannot be excluded, especially if dominant TEOAE frequencies are close to each other.[14]

The frequency of the emission controls the specific latency of the TEOAEs recorded in the ear canal. For example high frequency stimulation elicits TEOAEs with shorter latencies compared with those evoked by low frequencies.[22] It may not be possible using a complex nonlinear response such as TEOAEs to determine precise measurements of the latency.[14]

The psychoacoustic detection threshold is often higher than the corresponding detection threshold of the TEOAE.[14] These observations are consistent with the notion of a mechanical, preneural origin for TEOAEs.[14] However, the visual-detection threshold is influenced by the frequency content of the TEOAEs. Therefore,

ears which exhibit narrow frequency components in their TEOAEs clearly have lower thresholds than ears without such components.[24]

The amplitudes of TEOAEs depend on stimulus level as well as on the number and frequencies of innate, dominant emissions. Moreover, emission amplitudes also depend on the frequency response of both the middle ear and the recording system.[25] In addition, factors that are presently unknown, but are specific to individual ears (e.g. cochlear resonances), are also likely to contribute to TEOAE magnitudes. However, as in the vast majority of cases TEOAEs are composed of multifrequency responses, methods that integrate the emitted response within specific time windows or power spectra have been used frequently to estimate TEOAE amplitude.[25]

Considering these methodological difficulties, details of the response/growth input/output (I/O) functions reported in studies vary considerably.[22] The Input/Output function is the relationship, expressed graphically, between the stimulus intensity (amplitude in dB) and the amplitude of the response (in dB). [26] Awareness of normal and pathological I/O functions is helpful when interpreting responses (e.g. estimating threshold) and measuring an individual's I/O function can also be helpful as an indicator of recruitment (an aspect of certain forms of deafness wherein the growth of loudness of sound of increasing intensity is greater than in normal ears).[2, 26] The first I/O function for TEOAEs was documented by Kemp (1978), who related the amplitude data to the square root of the stimulus level. The graph of Kemp's published plot showed an almost linear growth, up to a stimulus level of about –25 dB SPL/Hz, (this corresponds to about 13 dB HL), along with strong saturation above this level of stimulation.[14] Other researchers have also shown that below stimulus levels of 10-20 dB HL there is constant growth of the TEOAES with a pronounced saturation above this level (Wit and Risma, 1979; Kemp and Chum, 1980; Wilson, 1980; Schloth, 1982; Zwicker, 1983).[14] Zwicker (1983) discovered that I/O functions, with the characteristics mentioned earlier regarding linear growth and pronounced saturation, were exhibited in the majority of cases in ears in which SOAEs could not

be detected. This may mean that at low stimulus levels, spontaneous emissions interfere nonlinearly with TEOAEs so that linear growth, for example , would be unlikely in ears with SOAEs.[14] There is a general consensus that nonlinear growth of TEOAEs occurs for stimulus levels >20-30 dB SL. Saturation with increasing stimulus levels is one of the most distinct characteristics of TEOAEs and is frequently used as a means of extracting TEOAEs from the ear canal signal.[14] The practical importance of the nonlinear growth and saturation properties has been recognised in the design of commercially available equipment that makes use of linear cancellation technique.[27] Precise details concerning the slopes of growth functions for TEOAEs at lower stimulus levels and there importance in practical terms is less clear.[14]

1.2.2.3) Stimulus Frequency Otoacoustic Emissions (SFOAEs)

SFOAEs are responses recorded to a continuous tone. The stimulus and the emission overlap in the ear canal; therefore, the recording microphone detects both. Interpretation depends on reading a complicated series of ripples in the recording.[11] Currently, SFOAEs are not used in clinical practice.

1.2.2.4) Distortion Product Otoacoustic Emissions (DPOAEs)

The DPOAE is a faint tone, about 0-20 dB SPL, generated when the cochlea is stimulated simultaneously by two other pure tone frequencies presented to the ear, whose ratio is between 1.1 to 1.3, that is detected in the external auditory meatus (**Figure 1.5**) .[25, 28]

DPOAEs are produced by the OHCs of a normally functioning cochlea and are an indicator of a healthy cochlea. If DPOAEs are absent this suggests abnormal cochlear function. Research on the generation mechanism of DPOAEs has highlighted the presence of two important components in the DPOAE response, one generated by an intermodulation “distortion” and one generated by a “reflection”.[28] DPOAEs are present in 100% of normal adult ears. Age effects the DPOAE responses by lowering

the DPOAE amplitude and narrowing the DPOAE response spectrum (i.e. responses at higher frequencies are gradually diminishing).[28]

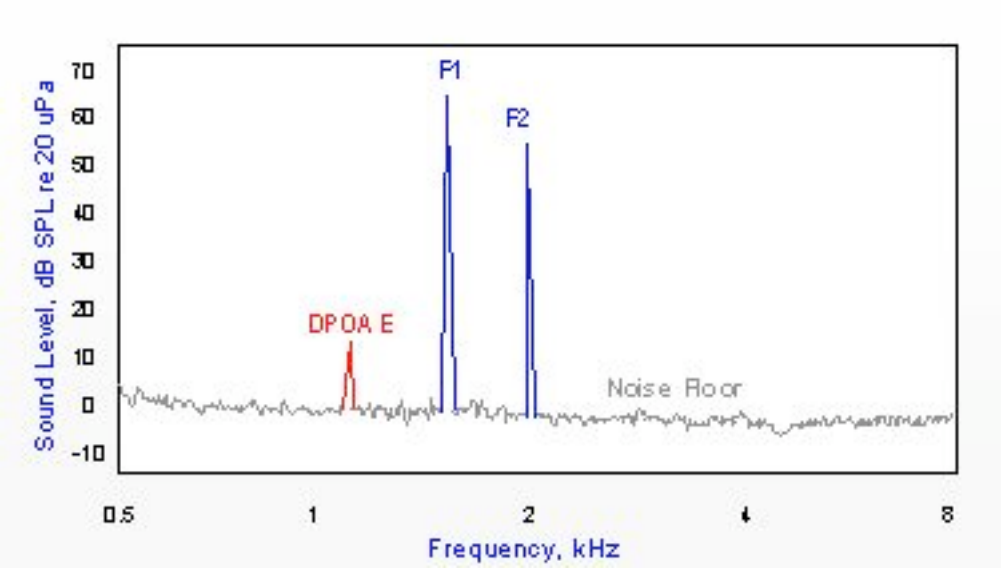


Figure 1.5. *Sound Spectrum in Occluded Ear Canal shows three characteristic peaks above the Noise Floor: Primaries F1 (typically presented at 65 dB SPL) and F2 (typically 55 dB SPL), and DPOAE (typically within 0-20 dB SPL in normal ears). DPOAEs are most prominent at FDP = 2F1 - F2. Being very faint, DPOAE is typically detected by averaging and Fast Fourier Transform (FFT) of the signal. This process is time-consuming and provides only static value of DPOAE amplitude.[28]*

Pure tones which stimulate the cochlea are called primaries and they are denoted as F_1, which is usually the lower tone and F_2 which is commonly the higher tone. Their corresponding amplitudes are assigned as L_1 and L_2.[28] In order to generate the intermodulation DPOAE component, the primaries in general have frequencies which are close to each another. The ratio of the F_2 / F_1 frequencies is known as the frequency ratio and denoted F_R. The F_R has an effect on the amplitude of the DPOAEs at different frequencies.[28] As a result of intermodulation the cochlea generates a long series of components which do not exist in the input stimuli; these components are the distortion products. The most prominent and frequently used in clinical practice is denoted as $2F_1 - F_2$. This is the cubic difference distortion product.[28] DPOAE protocols employed in clinical practice are divided into two groups, those which use primaries with equal intensities are called symmetric ($L_1 = L_2$), for example 70-70 dB SPL and those which use unequal primary intensities ($L_1 >$

L_2) are called asymmetrical, for example 65-55 dB SPL. The latter type are better in identifying patients with hearing impairments and are used in most screening programmes. [28]

The intermodulation components are generated close to the F_2 primary tone, when asymmetrical protocols are used, hence the DPOAE information is referenced to F_2. When symmetrical DPOAE protocols are used the information is referenced to the geometric mean, which is defined as the square root of $F_1 * F_2$.[28] The DPOAE information acquired can be presented in two different ways; the DP-gram modality (**Figure 1.6**) and Input-Output modality (IO modality). In the DP-gram the $2F_1 - F_2$ amplitudes are measured at various F_2 frequencies, having fixed the stimulus intensities, for example F_1=65 dB and F_2=55 dB SPL. In the Input -Output (IO) modality, the $2F_1 - F_2$ are measured at a fixed F_2 frequency, varying the primary stimulus levels.[28]

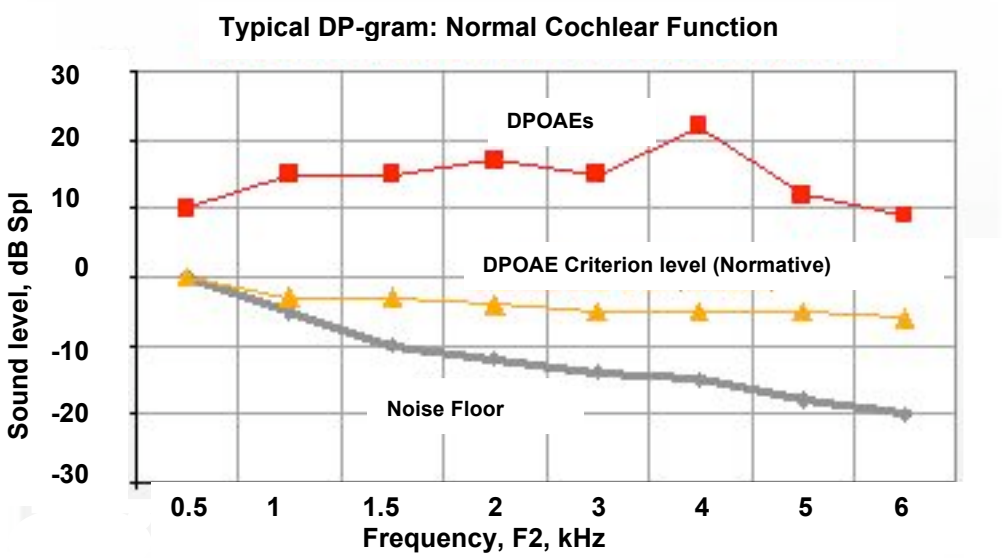


Figure 1.6. *The normal ear typically produces DPOAEs at the levels of 0-20 dB SPL across audiometric frequency range 500-8000 Hz. DPOAE Criterion Level (DPCL) separates normal and abnormal DPOAE levels, with DPOAE levels above the DPOAE Criterion Level considered normal. Noise Floor (NF) is the level of ambient noise around DPOAE frequency and typically decreases at higher frequencies. Signal-to-Noise Ratio (SNR) is the interval between DP-gram and the Noise Floor, typically exceeding 6-10 dB in normal ears.[28]*

Using the following model the mechanism of generation of DPOAEs can be explained. The cochlea can be considered as a black box and the ear-canal signal as representing the output of this system. Two pure tones are applied into this black box, which, traditionally, are referred to as the f1 and f2 primaries (f1<f2). If the cochlea response is linear, then the output frequencies would be the same as the input frequencies. [28] Thus, there would be a direct relationship of the input to the output signal graphically producing a straight line (for the I/O function), representing a linear function. If the function relating the input of the two sinusoids to the output is not a straight line, that is, the input/output (I/O) function is nonlinear, new frequencies will be generated at the output. I/O functions that are used to represent the basilar membrane (BM) response have been detailed by Fahey et al (2000).[29] One of these I/O plots is very similar in shape to the hair cell receptor voltage versus stereocillia displacement function measured earlier by Hudspeth and Corey (1977) and Russell et al (1986).[30, 31] These types of nonlinear I/O functions acquired from various cochlear structures are relevant to the discussion of physical mechanism(s) within the cochlea that are capable of generating DPOAEs. If these functions exhibit both even- and odd-order symmetry, then all the DPOAEs that can be found in the ear-canal signal will be observed. [28] Thus, combinations of the primaries that result in even-order DPOAEs, such as the simple difference tone, f2-f1, and many odd-order DPOAEs, the largest and most commonly studied one being the 2f1-f2 frequency, will be recorded. Other DPOAEs seen are the lower odd-order sideband 3f1-2f2 and the upper odd-order sideband DPOAE at the 2f2-f1 frequency.[28]

When the f1 and f2 primaries are presented to the ear canal, the first constraints that must be placed upon DPOAE generation can be appreciated from observations of the underlying BM mechanics. Presentation of a pure tone to the external auditory meatus causes the well-known travelling wave of displacement on the BM. This peaks at its characteristic frequency (CF), and then rapidly fades out at more apical points of lower frequency. The pattern of displacement defines the place on the BM where DPOAEs must be generated. That is, the only location where f1 and f2 can mix in the nonlinearity (commonly assumed to be based in the OHCs) is in the tail of the

BM displacement of the f1 primary. On placing f1 at a much higher frequency, f2 cannot substantially interact with f1, because of the steep apical cut off of BM displacement. Thus, in theory, DPOAEs must be produced at, or near to, the f2 place, where the two primaries can physically interact on the BM.[28]

This theoretical prediction is borne out by findings from suppression studies in which a third tone (f3) is used to interfere with the generation of the DPOAE. By sweeping f3 in level and frequency, it is possible to produce suppression tuning curves (STCs), with their tips characteristically tuned near the f2 place for the 2f1-f2 DPOAE.[28, 32] Much of this requirement can explain the much studied f2/f1 ratio effect, in which the level of the DPOAE decreases on either side of an optimum ratio value. The optimal f2/f1 ratio is approximately 1.22 in humans, and DPOAEs are of the greatest magnitude at this ideal separation of the two primary tones. As the primary f1 and f2 tones come closer together, some of this ratio effect, may be accounted for by mutual suppression or interaction of multiple DPOAEs.[33] This phenomenon may also be the result of a second-filter effect (noted by Brown et al, 1992).[28]

When DPOAEs are produced in the cochlea, they are seen on the BM, and they propagate, as if they were external tones applied to the ear canal.[34] This ‘cubic-distortion tone’ can be heard by those of normal hearing, as the 2f1-f2 is lower in frequency than the f2 place where it is generated. This happens because the 2f1-f2 DPOAE travels to its characteristic place, where it then acts like an external tone.[28]

DPOAEs are more effectively produced at lower primary-tone levels, when the level of f2, i.e. L2, is lower than the level of f1, i.e. L1, and this can be explained by basilar-membrane mechanics. This is the familiar unequal-level primary tones protocol, typically 65/55 dB SPL, that is almost universally advocated in the clinical literature for measuring DPOAEs in human subjects.[33] The rationale for lowering L2 is to equate the amplitudes of vibration of the travelling waves representing the two primaries, where they interact with each other on the BM. Consequently, as the BM response is highly compressive at the CF, assumed to be f2 for DPOAEs, and linear at the off-CF

frequency of f1, then lowering the level of f2, where it is 'amplified' at low stimulus levels, helps to equate the two stimuli, where they interact at the f2 place. As primary-tone levels increase, this L1-L2 difference is no longer required to equate the two stimuli. [28, 35]

In summary, DPOAEs are produced when the primary tones interact on the BM to stimulate nonlinear elements in the cochlea. The OHCs are almost certainly the site of this nonlinearity.[36] Specifically, OHC electromotility, first described by Brownell et al (1985), is the proposed source of the cochlear amplifier, described in more detail earlier (see **Section 1.1.2**).[36] It has been postulated that the OHC electromotility-based cochlear amplifier is responsible for the compressive BM response at CF, and the associated sharpness of nerve-fibre tuning seen in physiologically healthy preparations, but absent in damaged or dead animals, along with the nonlinearity responsible for producing DPOAEs.[34]

DPOAEs probably originate from a variety of nonlinear sources, besides OHC electromotility, that participate in the OHC-transduction process including opening and closing of transduction channels (Patuzzi 1998), nonlinearities in stereocillia-bundle motion (Jaramillo et al 1993), and asymmetries in stereocillia stiffness.[28, 37]

Related to the question of the generation mechanism of DPOAEs is the issue of where do DPOAEs originate from with respect to a point(s) along the cochlear partition. The generally assumption is that DPOAEs come from the f2 place, detailed above. However, once produced, they also propagate as travelling waves along the BM. As a result of this, it is possible for a propagated DPOAE to stimulate the DPOAE place, i.e. the 2f1-f2 frequency place, where other OAEs can be further produced by the mechanism of linear-coherent reflection.[38, 39] These two sources (i.e. the DPOAE generated at the f2 place and the emissions reflected from the 2f1-f2 DPOAE place) then mix to form the final ear-canal signal recorded.[28] There is also evidence for basal DPOAE sources that may also contribute to the final DPOAE signal. These basal sources are revealed as secondary regions of suppression or enhancement

above f2 during the recording of the STCs.[28] Such regions of suppression/enhancement have been observed at frequencies that are more than an octave above f2 (Martin et al 1999; Mills 2000), where it is unreasonable for the f3 to affect the f2 place, due to the steep apical cut off of the travelling wave.[28] A proposed explanation for these phenomena is that a harmonic of f1 (i.e. 2f1) interacts with f2 resulting in a simple difference-tone DPOAE.[28] This emission will always have the same frequency as the 2f1-f2, so, depending upon the phase of the difference tone, either suppression or enhancement could result.[28, 29] Another suggested possibility is that f3 acts as a catalyst to produce difference-tone DPOAEs by more complex routes that can then interact with the 2f1-f2 DPOAE.[28]

The upper sideband 2f2-f1 DPOAE appears to originate from its characteristic place on the BM.[32] This finding contrasts with the notion that all DPOAEs must be generated at the f2 place, where the two travelling waves representing f1 and f2 ideally interact. A possibility put forward is that the 2f2-f1 observed in the ear canal comes mostly from a difference-tone DPOAE based upon the interaction of a harmonic of f2 (i.e. 2f2) and f1, which is at the 2f2-f1 frequency.[32]

‘Active’ versus ‘passive’ DPOAEs have also been described. This conceptualization originated from earlier studies like Norton and Rubel (1990) and Whitehead et al (1992) in gerbils and rabbits.[40, 41] In these studies, low-level DPOAEs were eliminated by the administration of loop diuretics, such as ethacrynic acid or furosemide, while DPOAEs evoked by high-level tones remained relatively unaffected. These results lead to the notion that DPOAEs evoked by high-level tones were not relevant to cochlear function, and many clinical investigations focused on low-level primaries in the 55- to 65-dB SPL range. However, early research in humans clearly indicates that 75/75 dB SPL level primaries can accurately track the pattern of hearing loss in individuals with a hearing loss.[42] Studies in mice with age-related hearing loss indicate that all levels of primaries accurately follow the progressive degeneration of high-frequency OHCs observed in these animals.[43] Similarly, brief exposure to damaging noise levels adversely affects, not only low-level

DPOAEs, but also high-level DPOAEs.[44] More recent propositions are that there are not two sources of DPOAEs, that is, a low-level ‘active’ one along with a high-level ‘passive’ source. Rather, low-level DPOAEs are based upon a functional cochlear amplifier, whereas high-level DPOAEs arise when stimulation is sufficient to move the BM without amplification, in turn, stimulating remaining nonlinear elements to evoke DPOAEs.[28]

External factors that have demonstrated an effect on DPOAE production include aspirin and acoustic suppression.[14]

It is well known that, within an individual ear, SOAEs, TEOAEs, or SFOAEs, influence DPOAE amplitudes.

1.3) Normative properties of OAEs

1.3.1) The effects of gender and ear side on conventional OAEs

There is evidence for a peripheral lateralisation of the auditory system as well as the existence of a sex difference in the auditory periphery. Axelsson and Lindgren (1981) showed in a study of hearing thresholds in 139 classical musicians 88 musicians showed asymmetry of hearing between the ears greater than 15 dB at one frequency.[45] Of these subjects the left ear was worse than the right in 52. Hearing surveys have also shown the right ear to have a slightly greater acuity than the left ear. Tinnitus more often affects the left ear than the right.[46] Moreover, the average temporary threshold shifts after binaural exposure are higher in the left ear than in the right ear.[47, 48] Johnson and Sherman (1979) found differences in middle ear function as reflected by acoustic reflex thresholds; with the right ear averaging 3-7 dB lower threshold, thus more sensitive than the left ear.[49] The observation of a greater tone decay in the right ear than the left ear has also been made.[50]

Newmark et al. (1997) also showed that click evoked otoacoustic emissions (CEOAEs) differed as a function of gender and ear, the right ear responses were larger in amplitude at nearly all of the analysed frequencies.[51] In almost 75% of the males and 65% of the females, the right ear emissions were stronger than the left. In an investigation of 7- 49 year olds, Talmadge et al. (1993) found the ear side effect to be greater in male than in female subjects (this ear side effect being the greater prevalence of SOAEs in female subjects and for males to have fewer emissions from their left ears), although a study by Collet et al. (1993) revealed no correlation between SOAE prevalence and ear side in adults.[52, 53]

Kei et al. (1997) indicated a significant difference in the signal-to-noise ratio (SNR) of TEOAEs across sex, with females showing a higher SNR.[54] Furthermore, the right ear was found to have higher values in 'reproducibility' and 'response level' than the left ear. Females were shown to produce TEOAEs of greater amplitude than males; however no frequency/sex interaction occurred. Results obtained from newborns, using transient evoked otoacoustic emissions testing, also indicated significant differences due to sex, females being more sensitive than males with the differences in hearing sensitivities increasing as the frequency increased.[55] The greater hearing sensitivity in females and in right ears appears to parallel the abundance of spontaneous otoacoustic emissions (SOAEs) found in females and in right ears in both adults and full term neonates.[56] SOAEs are regarded as epiphenomena of micromechanical processes in the cochlea; which, once generated in the inner ear; exit the hearing system retrograde to the physiological sound path through the middle ear and are radiated to the external ear canal.[57] They are recorded with the use of a small probe microphone. SOAEs are emitted unheard by their owners and the prevalence of SOAEs declines with increase in age. In their study of 267 infants Lamprecht- Dinnesen et al. (1998) also found the SOAE prevalence per ear was significantly higher in female than in male subjects, with the sex difference being more distinct in the first year of life.[58] The study undertaken by Newmark et al. (1997) also showed that CEOAEs were significantly larger in females than in males.[51] DPOAE Responses from the left and right ears are often correlated (that

is, they are very similar). For normal subjects women have higher amplitude DPOAEs.[28]

1.4) Clinical applications of OAEs

OAEs are already an essential part of the audiological diagnostic test battery and are used in the newborn hearing screening programme and in the detection of non-organic hearing losses.

OAEs are regularly undertaken as part of neonatal screening and the Universal Newborn Hearing Screening programme (UNHS). Hearing loss in newborns can be an especially tragic affliction, for if it remains undetected, the child's speech and future intellectual development are impaired. The incidence of congenital profound hearing loss is 1-2/1000 newborns.[59-61] In the past the screening method of choice for "high risk" babies was ABR which as well as suffering from some fatal design flaws and expense, the test required well-trained personnel to perform it and interpret the data. It took up much time and resulted in a moderate amount of false positives. In addition, the definitions of "high risk", which encompassed everything from low birth weight and intrauterine infections to ototoxic drug exposure, missed about one-half of the children with hearing loss, including both conductive and sensorineural hearing losses.[59, 60]

The application of OAEs to infant screening began in Denmark in 1982, moved to France in the late 80s, and then to the USA where OAEs helped advance a national movement towards universal newborn hearing screening. Austria achieved near universal hearing screening in the late 90s. In the UK OAEs were deployed in UNHS programmes in a few key centres from the late 80s, but since 2002 has the UK been committed to a new national newborn hearing screening programme. The NHS Newborn Hearing Screening Programme completed a phased roll-out process across the country in 2006 and at present approximately 124 sites offer hearing screening for newborn babies.[61] Currently more than 1,700 babies are screened daily as part of

the NHS Newborn Hearing Screening Programme.[61] The only countries in the world with universal newborn hearing screening are Wales, Northern Ireland, Scotland and England.[61] So far it is estimated that in England 1675 children with confirmed bilateral deafness and 858 children with confirmed unilateral deafness have been identified by the newborn hearing screen, thus enabling the necessary help.[61] National programmes are being considered in many European countries including Netherlands, Spain and Poland.[59, 60]

Otoacoustic emissions (OAEs) offer a cheap, easy, quick, and relatively accurate alternative to ABRs, or a complement to ABRs (a two-stage OAE/AABR screen being frequently used). OAE testing does not require a soundproof room, is well tolerated by children, and does not require extensive training to perform or interpret. Due to their requirement of a functioning middle ear (ME), OAEs can also be used to detect ME dysfunction. Thus the two main causes of hearing loss in the newborn, ME dysfunction and sensorineural hearing loss, are unearthed by this procedure. Unfortunately, these two causes are not distinguished from one another by OAE testing: OAEs can act as a detector, pointing to the need for further investigation, as is the function of any screening test. In addition, several large studies such as the Rhode Island Hearing Assessment Program, have refined a two-stage screening process (if they fail the first stage screen, re-test in 4-6 weeks and then refer if failing again) which helps eliminate the high number of false positives that have been historically associated with TEOAE screening. Cost analysis by this same group found that TEOAEs testing, using the two-stage process, is economical, costing around $4000 each baby detected with a hearing loss, or around $20 a head for all comers.[62]

The Wessex study has shown that OAEs improve in the days following birth, this has been described in **section 1.2.1** and **Figure 1.7** displays one of the findings of this study.[63]

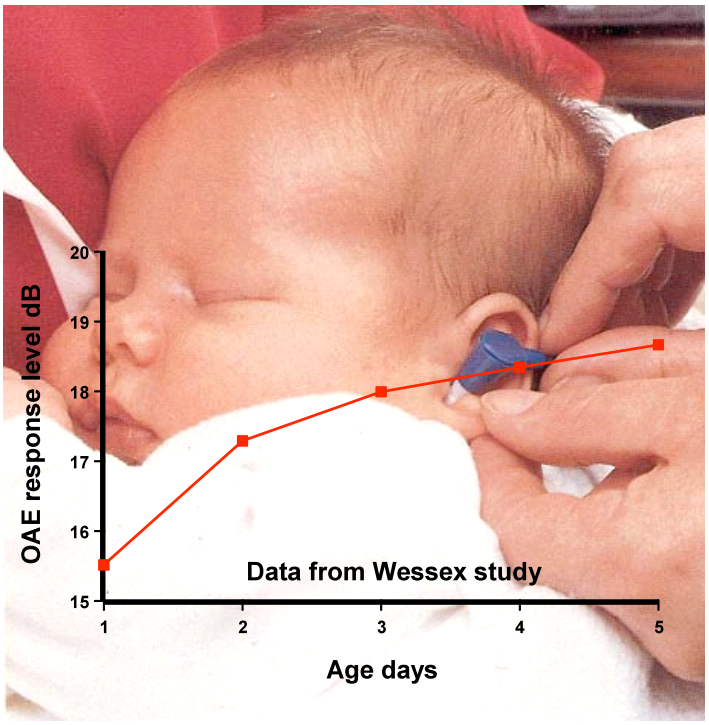


Figure 1.7. *Neonatal testing and data from Wessex region. The OAE response level increases with age days following birth. (Reproduced with permission from Professor ARD Thornton of MRC IHR, Southampton).*

In difficult-to-test patients, transient evoked otoacoustic emissions provide an alternative objective method of assessing the peripheral auditory system.

As mentioned earlier, TEOAEs can be used to detect non-organic hearing losses, as they can be obtained quickly and accurately in the outpatient setting, and provide a non-invasive, objective measure of cochlear function. Their presence suggests a hearing threshold of about 30 dB HL or better.

The clinical use of TEOAEs in children with autism has been described by Grewe et al, 1994.[64] In their study they were able to record TEOAEs in 9 out of twelve ears, of six children. The difficulty in obtaining emissions from the other three ears was due to lack of subject co-operation.[64] In their study they did, however, realise that

testing in this potentially non-compliant population required longer, usually between 10 and 30 minutes.

Otoacoustic recordings have been made in patients with both noise-induced hearing loss (NIHL) and ototoxicity. As both of these sensorineural disease processes affect primarily the cochlea's OHC population, OAEs are superb for detecting their effects. OAEs objectivity is particularly useful for legal battles involving NIHL and worker's compensation. Also, OAEs can be used to monitor patients for ototoxicity due to certain drugs, such as chemotheraputic agents or antibiotics. In fact, several studies have found that OAEs detect ototoxicity before behaviour audiograms, or detect a more severe deficit than ABRs, implying that OAEs are more sensitive to hearing loss due to these OHC-specific pathologies. **Figure 1.8** shows both an audiogram and a DP-gram for NIHL.

OAEs may also be used to monitor dynamic pathologies. Any process that affects cochlear function differentially over time can be monitored for change so that the physician can make a fully informed decision regarding treatment options, as well as keep the patient aware of their disease status. An example of this is Ménière's disease, which is characterized by a fluctuating hearing loss. OAEs can be used not only to monitor the changes in the long term but also to evaluate the efficacy of treatments in the short term, such as urea or glycerol.

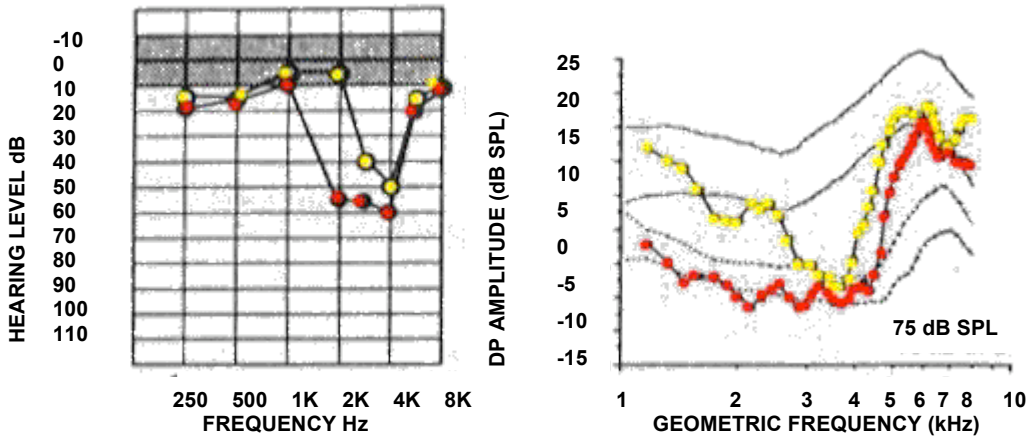


Figure 1.8. *Showing an example of the correlation between OAE's and traditional behavioural audiograms. Both show a 4 kHz deficit in hearing. The x-axis in each plot represents the frequency tested. On the left, the audiogram's y-axis represents the hearing threshold, i.e., the softest sounds the patient can hear. On the right, the y-axis of the DP-gram (testing distortion product otoacoustic emissions) represents the OAE output of the cochlea tested (so, the higher the amplitude, the better the hearing). Dips in the graph patterns represent the deficits.[65]*

Other disorders which also progress toward decreased function, such as presbyacusis, can be monitored for changes with OAEs, which can also be used to aid in the diagnosis of the causes of hearing difficulties. **Figure 1.9** illustrates a high frequency loss.

Perhaps one of OAEs potential clinical strengths is in the ability of OAEs to aid in distinguishing between cochlear and retrocochlear (i.e., "behind the cochlea" - anywhere in the auditory pathway from the spiral ganglion to the central nervous system) lesions. Because OAE generation hinges on a properly working external ear, tympanic membrane, middle ear (including ossicles) and cochlea circuit; the presence of normal OAEs implies that these structures are intact. This explains why OAEs can be used to evaluate middle ear function in addition to cochlear function. Actually, OAEs are doubly dependent on a functioning middle ear, as not only the sound signal, but the cochlear response both must traverse this physiological entity. A

perfect illustration of this localization is the evaluation of hearing loss suspected to be from an vestibular schwannoma (VS), which is a tumour of the nerve sheath cell of the eighth cranial nerve, which is responsible for innervating the vestibular apparatus and cochlea. These present typically with tinnitus and a progressive, unilateral, high-frequency hearing loss, as well as with disequilibrium in about half the cases. Currently, ABR is used in their diagnosis, but its disadvantages compared to OAEs advantages have already been covered above. However, the “gold standard” for appraising VS’s probability is a thin section MRI with contrast. OAEs may be used as an adjunct to the MRI. Generally, if a patient exhibits a hearing loss as well as normal DPOAEs, the pathology is most likely retrocochlear, although having abnormal DPOAEs with hearing loss does not ensure a purely cochlear aetiology.[66]

Additional uses for OAEs continually arise as more clinical research is performed with them. One such possible future use involves monitoring cochlear blood flow during intra-cranial surgery on the eighth nerve, such as vestibular schwannoma surgery (**Figure 1.10**).[67]

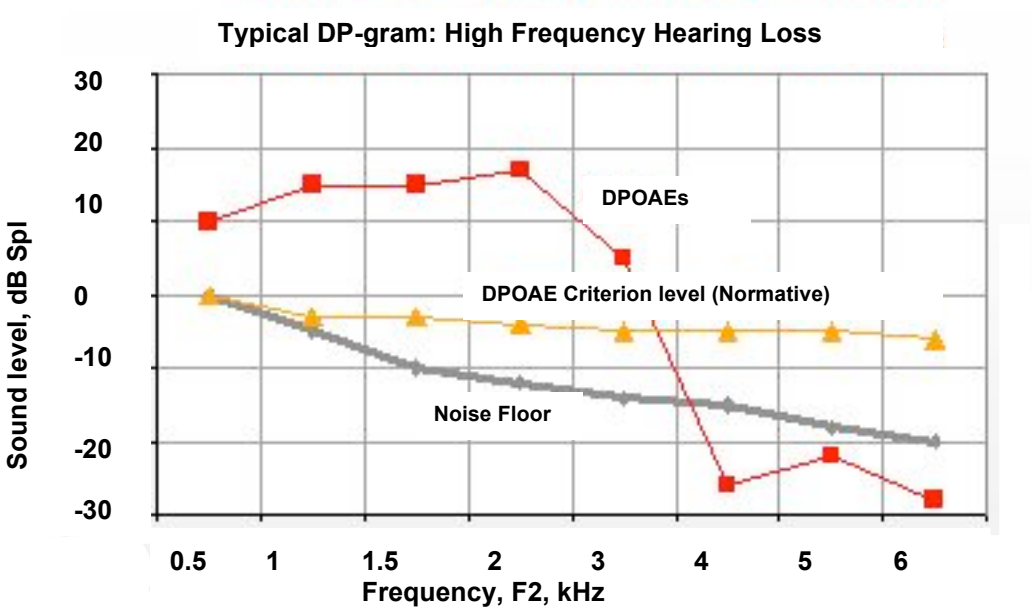


Figure 1.9. *Hearing disorders diagnosed more precisely using DPOAEs in the battery of audiological tests. Cochlear dysfunction results in significantly lower DPOAE levels or the absence of DPOAEs, which is indicated by DP-gram points below the Noise Floor (reprinted with permission).[28]*

Auditory neuropathy and central auditory dysfunction may exhibit normal DP-grams indicating normal cochlear function. Ototoxic drugs and occupational noise cause deteriorating of DPOAEs, often before pure-tone thresholds. Therefore DPOAE testing is a good tool for detecting and monitoring such conditions. Hearing Screening is fast and effective. Malingering can be detected objectively.

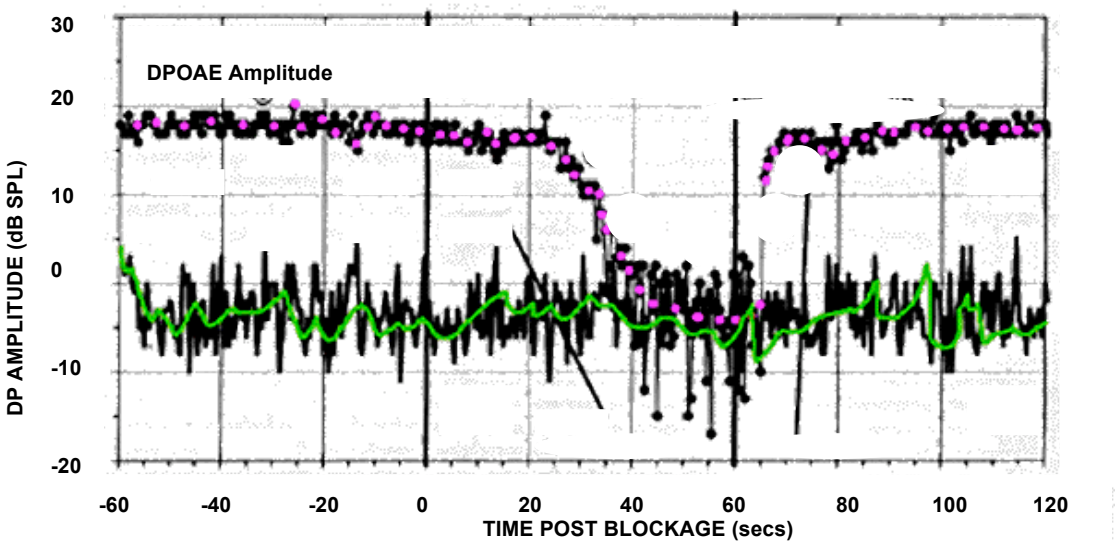


Figure 1.10. *This is taken from Widick et al. 1994. It shows the changes in the DPOAEs when blood flow to the cochlea is interrupted and then restored. The DPOAE amplitude drops almost immediately (within 20-30 seconds) after compression, while its recovery is even faster. This research supports the use of intraoperative monitoring of DPOAEs.[68]*

1.5) Recording methods: Maximum Length Sequences (MLS)

A maximum length sequence (MLS), in its audiological application, may be represented by a quasi-random train of clicks and silences. Mathematically these may be designated as a sequence of ones and zeros, with each one giving rise to a click and each 0 to a silence.[69, 70] Such a sequence may be generated by a shift register with an exclusive OR gate on two or more of its bits, which is fed back to the input.[69] If the seed value in the register is zero then the output will be always zero. This is a minimum length sequence. Therefore, eliminating the zero case, a maximum length sequence has a length of $2^n - 1$ where n is the number of bits in the register.[69] The bits which are selected to feed into the exclusive OR gate are known as taps and the position of these taps is critical. If the taps are not at the proper placings then the sequences produced may not be of the maximum length but can be of a shorter length than $2^n - 1$ before repeating.[69]

The point of using such a sequence is that it enables responses to be recorded at high stimulation rates where the time between stimuli is significantly less than the duration of the response. For example, an MLS with a minimum time between clicks of 2 ms may be used to record a response lasting 20 ms. It is the mathematical properties of an MLS that enable the long duration response to be deconvolved from the overlapped set obtained in response to the MLS stimuli.[69]

Detailed information about the deconvolution involved with MLS has been published.[71] Eysholdt and Schreiner (1982) first reported the application of MLS in the audiological field when they described its application to recording the auditory brainstem response (ABR).[72] Later on the ABR obtained using MLS techniques has shown the presence of nonlinear temporal interaction components in addition to the more familiar, linear component.[69]

When utilising click EOAEs a number of response epochs must be averaged to improve the signal-to-noise ratio, thus producing a clear waveform. However, the maximum click presentation rate is limited by the window or epoch length; the window normally being of the order of 20 ms and so the maximum stimulation rate is about 50/s.[69] If the click presentation rate was increased in order to shorten the test duration, the responses would overlap each other and the stimulus clicks and corrupted waveforms would ensue. This problem can now be overcome, as EOAEs can be recorded using maximum length sequence (MLS) techniques. The Medical Research Council Institute of Hearing Research at Southampton has developed a new EOAE technique (MLS OAE), which enables stimulus rates of up to 5000 clicks/s to be used (**Figure 1.11**).[73]

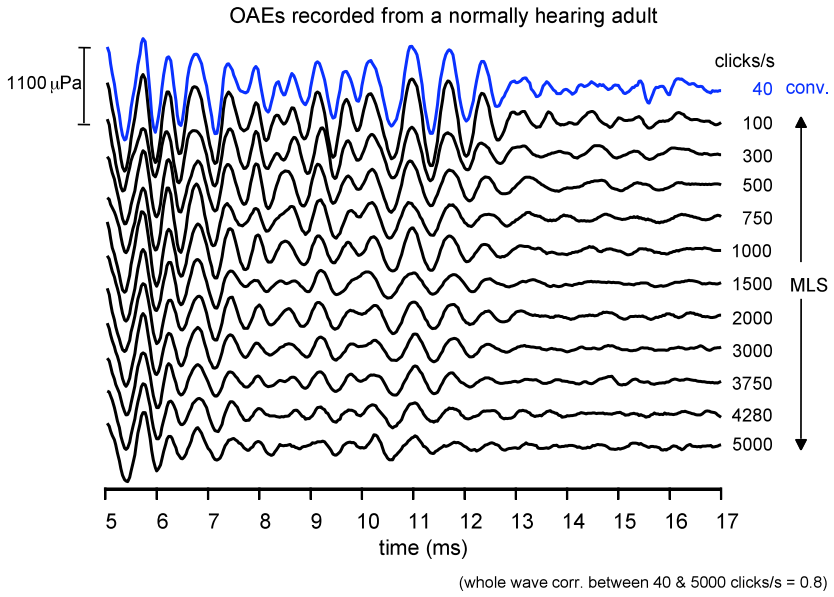


Figure 1.11. A conventional OAE (obtained at 40clicks/s) and MLSOAEs.

The equipment consists of standard DSP boards and computers. This technique allows considerable reduction in recording time and a greater range of stimulus rates compared with conventional recording. The gain in signal-to-noise ratio obtained by stimulating at rates of up to 5000 clicks/s enables this technique to detect responses that are only 20% of the amplitude of the responses that are detectable by the conventional technique in the same recording time. Previous studies of MLS transient evoked otoacoustic emissions (TEOAEs) in adults have shown that there is a decrease in emission amplitude with increase in stimulus rate.[74, 75]

1.5.1) Advantages of the MLS technique

One of the very practical problems of using evoked otoacoustic emissions (EOAEs) to test neonates and young children is that, to obtain a good recording, responses must be averaged over a period of a minute or so; the child or baby must therefore be quiet for that length of time.[76] To achieve this quiet period can take

many more minutes of testing because the equipment will reject sweeps that are contaminated by noise or movement artefacts.[73] This problem may be addressed by decreasing the test time. In addition to this, if neonates are tested within the first three days of life, then the overall failure rate is unacceptably high. Thornton et al (1993) have shown that with 50% of babies being discharged within the first three days, some 36% of normally hearing babies will be expected to fail evoked emission testing using conventional equipment.[73] It seems probable the emission is present but at a much reduced amplitude in the first three days of life. Therefore, extrasensitivity of the testing technique is desirable.[73]

1.5.2) Normative Properties of MLSOAEs

Normative data on the relationship between TEOAEs recorded conventionally (at 40 clicks/s and those recorded using the MLS technique (between 100 and 5000 clicks/s) have been provided by Hine and Thornton (1997).[77] MLS averaging was performed at 11 rates 100, 300, 500, 750, 1000, 1500, 2000, 3000, 3750, 4280 and 5000 clicks/s. For each subject the highest click level used was 68 dB peSPL, this stimulus level was dropped by 5 dB down to 38 dB peSPL. They showed that the waveform morphology and input/output function with latency pattern was similar conventional and MLS TEOAEs. The single major difference between TEOAEs recorded at varying rates was in their absolute amplitude. On increasing the click rate from 40 clicks/s there was a reduction in amplitude that almost resulted in an asymptote at approximately 1500 clicks/s. This was expressed as a percentage reduction in the amplitude compared with that recorded conventionally at 40 clicks/s, and it was shown that this MLS ‘rate effect’ was independent of stimulus level over all but the lowest test level 38 dB peSPL. They concluded that over a wide range of amplitudes of conventionally recorded TEOAEs the mechanism involved in the MLS rate effect performed in a way that reduced the amplitude in a near constant proportion, regardless of the original size.[77]

MLS TEOAEs have been shown to be the most sensitive indicator in the early identification of noise-induced hearing loss.[78]

1.5.3) Objective 1

Conventional EOAE amplitude differs between ears and sexes; female subjects having responses of greater amplitude than male subjects and right ears larger responses than the left. As a pre-requisite to clinical use it is necessary to establish if these differences occur with the MLS OAE technique and whether they change with stimulus rate.

1.6) Otoacoustic emission nonlinearity

1.6.1) Origin of OAE Nonlinearity

The mammalian cochlear response is nonlinear in healthy animals.[79] Increasing the magnitude of stimulation does not always produce a proportional increase in the velocity or displacement of basilar membrane (BM) vibration.[79] For high characteristic frequencies the response is nonlinear for frequencies close to the characteristic frequency, but linear for frequencies an octave below the characteristic frequency.[79] There is much evidence that this mechanical nonlinearity originates predominantly in the processes of mechanoelectrical and electromechanical transduction in the outer hair cells.[80] As these processes are central to the functioning of the cochlear amplifier, this raises the possibility that the characteristics of cochlear mechanical nonlinearity may carry useful information about cochlear amplifier health.[80] The cochlear amplifier has been described in more detail earlier (**Section 1.1.2**).

Shera and Guinan (1999) presented a taxonomy for mammalian OAEs that can be experimentally verified.[39] In this conceptualization, Shera and Guinan (1999) proposed that OAEs arise from two fundamentally different mechanisms. [39] Thus,

there are OAEs that arise by linear reflection and those that are generated by nonlinear distortion. This distinction forms a ‘family tree’ of OAEs in which TEOAEs, SFOAEs, and SOAEs are based upon linear reflections, whereas DPOAEs are produced mainly from nonlinearities acting as emission sources.[81] This classification system is extremely useful in that OAEs can be categorized based upon their mechanisms of generation. Thus, the familiar click-evoked TEOAEs come from reflection off of pre-existing micromechanical impedance perturbations, distributed along the organ of Corti, which might include such conditions as disorganized outer hair cell (OHC) arrays (e.g. Lonsbury-Martin et al 1988), that are unique to each cochlea. Alternately, variation in the gain of the OHC active feedback process has also been suggested.[82] Such irregularities create reflections from multiple sites that sum with different phases. [82] Only those reflections that sum constructively and arise from the tip of the basilar membrane excitation pattern in an active cochlea will have sufficient amplitude to be recorded in the ear canal as an emission.[83] This reflection mechanism is often denoted as the ‘place fixed’ phenomenon.[84] On the other hand, DPOAEs arise primarily from nonlinear elements in the cochlea that are stimulated by the in-coming traveling waves. Thus nonlinear distortion arises from the action of the cochlear amplifier producing a wave-related mechanical interaction on the basilar membrane and depends on inherent physiological nonlinearities of the cochlear amplifier.[84] Because this mechanism is associated with the travelling wave, it has historically been called a ‘wave-fixed’ phenomenon[70].[85] What is most important to realize is that OAEs recorded in the ear canal, especially in humans, are rarely due purely to one form or the other, but represent a mixture of the two emission sources.

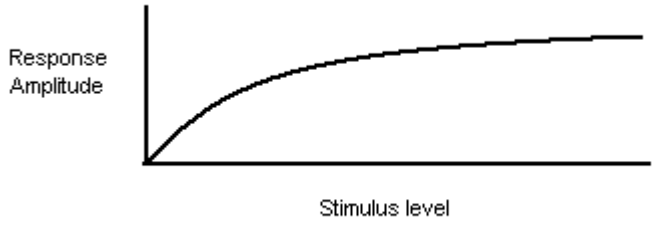
1.6.2) Types of OAE nonlinearity

Otoacoustic emissions exhibit a range of nonlinear phenomena. In the amplitude domain, the (I/O) input-output functions display nonlinear compressive characteristics.[86, 87] In the frequency domain, it is possible to demonstrate two tone suppression, and distortion product otoacoustic emissions are also a good example of nonlinearity in this domain.[86] As such it is unlikely that OAEs would be linear in the temporal domain.[87] Indeed, Delentre et al (1997) whom demonstrated temporal nonlinearities in the cochlear microphonic (CM), proposed the hypothesis that the temporal nonlinearities seen in the auditory brainstem response (ABR) data could have their origin within the cochlea at the hair cell level.[87, 88] In the temporal domain Volterra slices (VSOAEs) provide a measure of the nonlinearity of the system. **Figure 1.12** represents the different types of nonlinear distortion that can be found in OAEs. The I/O function of TEOAEs (CEOAEs) and DPOAEs have been described in **Section 1.2.2**.

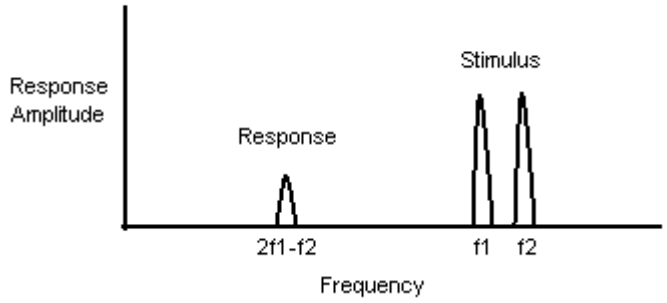
The I/O functions of TEOAEs recorded with conventional signal averaging techniques at stimulus rates of up to 50 clicks/s have been widely documented.[77] Kemp (1978) observed the amplitude of the response increased with increasing levels of stimulation. At low levels of stimulation the response was linear, but as the stimulus level increased the response was saturated.[77, 89] This compressive nonlinearity thus distinguishing the true TEOAE from the passive linear response.[77] Grandori et al (1993) detailed the relationship between the stimulus level and the latency of the response. They noted the compressive nonlinearities to be greatest at moderate to high stimulus levels and for the later parts of the response.[77, 90] Hine and Thornton (1997) compared the I/O functions of emissions recorded using conventional averaging at 40 clicks/s with those recorded using the MLS technique, at rates up to a maximum of 5000 clicks/s.[77] They showed that the form of the I/O function is essentially independent of rate, over the majority of levels tested.[77] The main difference between the rates was not the effect of changes in level, but how the rate itself affected the amplitude of the emission.[77] The increase in click rate obtained

using the MLS technique resulted in a suppression of the emission of the amplitude.[77] Picton et al (1993) suggested the effect of increasing stimulus rate may be related to nonlinear processes in the cochlea that determines the I/O function for the stimulus level.[77, 91] Picton and co-workers hypothesis was that if two stimuli are presented close together, the effect of the second stimulus on the OAE generators within the cochlea will be less than the first because the response would be occurring from a point in the saturating region of their response curve. In the study by Hine and Thornton (1997) there was an overall similarity between the I/O functions for different rates and the good correlation between conventional and MLS TEOAEs indicated the same cochlear event being recorded.[77] Hine and Thornton (2002) also suggested that the cochlear temporal nonlinearity was in part related to the nonlinear process that determines the compressive input/output level for stimulus level.[92]

AMPLITUDE Compressive input/output function



FREQUENCY Distortion Product OAEs



TEMPORAL (NLTIC) Distortion components present only at high stimulus rates

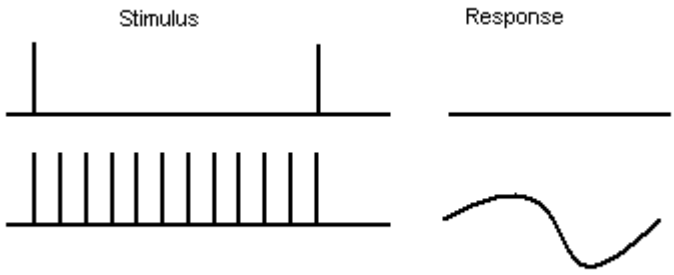


Figure 1.12. *Representation of the different types of nonlinear distortion that can be found in OAEs.[87]*

1.6.3) VSOAEs

Thornton et al examined whether temporal nonlinearities of the cochlear amplifier, as reflected by otoacoustic emissions (OAEs), exist and are distinct from any recording system non-linearities. Maximum length sequence stimulation, at various stimulus rates, was used to evoke OAEs from normally hearing subjects. Recordings from a 2cc cavity were also made. The data were analyzed to obtain the linear response and estimates of the slices of the 2^{nd} and 3^{rd} order Volterra kernels. This provided a measure of two and 3 click nonlinear temporal interactions, respectively. The results showed that temporal nonlinearities of OAEs do exist, are stable and repeatable within individuals and have properties that differ from those shown by the conventional linear response. Whilst some of the nonlinear response properties conformed to the expected pattern, of increasing amplitude with increase in stimulus rate, there are some areas in which they show an unpredicted complexity. Whilst system nonlinearities could be found, there was no difficulty in distinguishing between the physiological and system non-linear components. New areas of research and application may result from the use of these new OAE responses.[70]

Vito Volterra, born in 1860 in Italy, left a lasting legacy; his equations of nonlinearity have been applied to many systems. The problem of completely describing the system is equivalent to determining a set of multidimensional functions known as the Volterra kernels of the system.[93] The set of Volterra kernels characterise a nonlinear system in terms of an infinitely large family of responses to all possible trains of clicks.[93] A method of determining these Volterra Kernels is by measuring the response of the system to a maximum length sequence. Thornton et al, 2001 have demonstrated that these Volterra kernels can be successfully recorded in human subjects and are easily distinguished from artefacts of the measurement system.[87]

The volterra series is defined by the following equation:

$$y(t) = \int_{-\infty}^{\infty} h_1(\tau)x(t-\tau)d\tau + \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} h_2(\tau_1,\tau_2)x(t-\tau_1)x(t-\tau_2)d\tau_1 d\tau_2 + \int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} h_3(\tau_1,\tau_2,\tau_3)x(t-\tau_1)x(t-\tau_2)x(t-\tau_3)d\tau_1 d\tau_2 d\tau_3 + \ldots$$

where x(t) and y(t) are the input and output from the system. The variable, τ, is the range over which the integration is carried out for each of the elements, known as the Volterra Kernels, shown above.[94] For a linear (or first-order) system, all the kernels are zero except for the first order kernel, h_1, which equals the system impulse response function .[80] This first order kernel is represented by the MLSOAE which corresponds to the OAE obtained from conventional recordings.[94] h_2 ,the second order kernel is 3 dimensional (amplitude by time for click 1 by time for click 2) and represents the convolution of all possible nonlinear interactions created by pairs of stimuli. **Figures 1.13** & **1.14** illustrate the kernel slices for a second order volterra kernel. h_3, the third order kernel is 4 dimensional (amplitude by time for click 1 by time for click 2 by time for click 3) and represents the convolution of all possible nonlinear interactions caused by triplets of stimuli.[94] The difficulty of the volterra system is identifying higher order kernels in the output, this can be overcome using a variant of the MLS technique.[94]

Volterra kernels are continuous functions of time , whereas MLSs are discrete binary sequences with a maximum resolution in the time domain, corresponding to the minimal interval between time clicks.[94] For example at 5000 clicks/s, the resolution is 0.2ms, and so, samples of the kernel, called slices, can be obtained for values of τ equal to multiples of 0.2ms.[94]

Figure 1.13

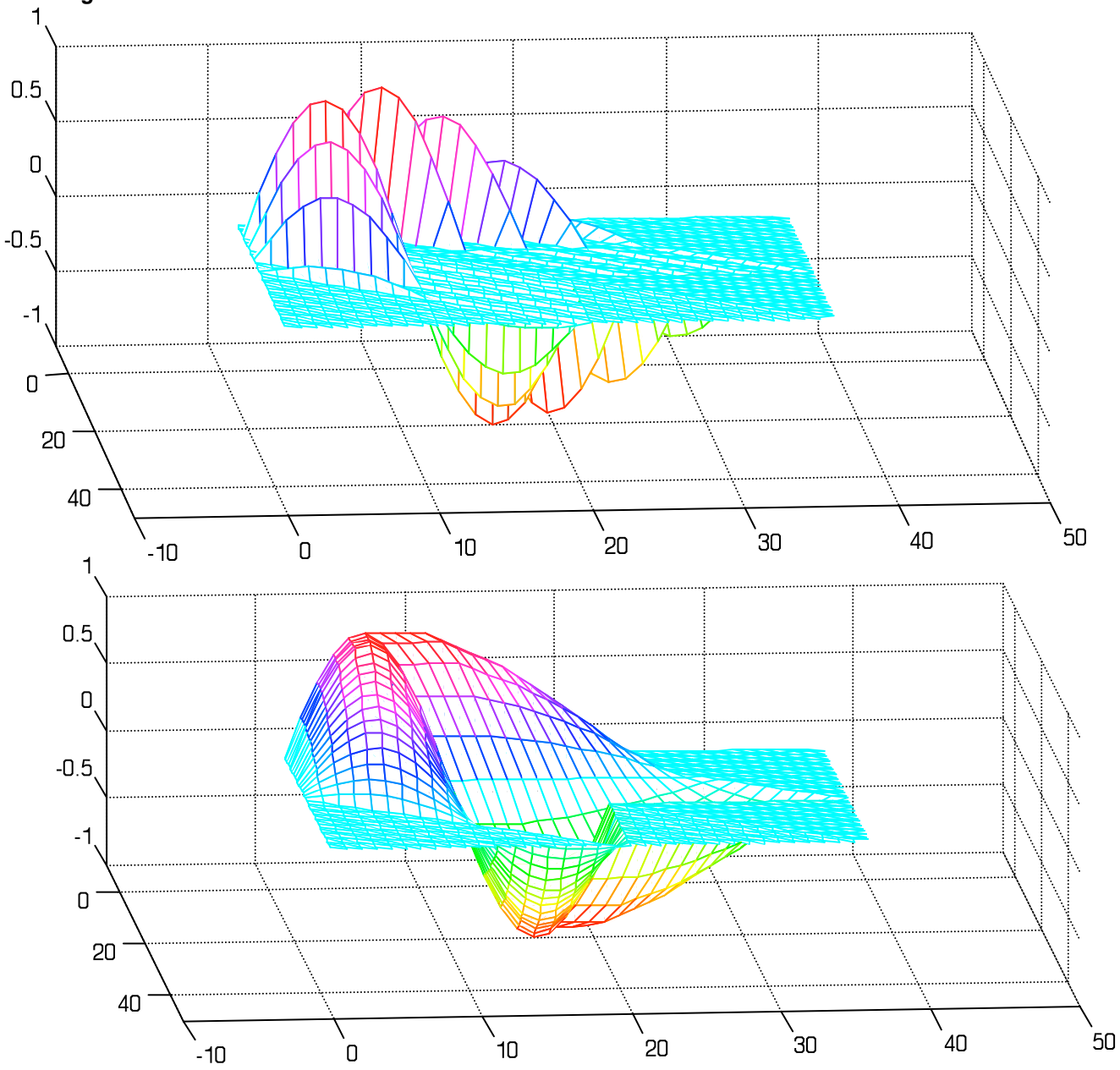


Figure 1.14

Figures 1.13 & 1.14. *Illustration for kernel slices for a second order volterra kernel. (**1.13**) The kernel slices that have been extracted from the deconvolved record. They run parallel to the main diagonal and are separated by the minimum time-interval between click stimuli. In **1.14** (lower figure), the slices have been interpolated to estimate the kernel itself.[94]*

1.6.3.1) VSOAEs normative properties so far

Normative data for Volterra slices over a range of click rates (from 1000 to 5000 clicks/s) and stimulus levels (56, 61, 66 and 71 dBpeSPL) recorded in 12 normally hearing adult ears have been demonstrated by Slaven and co-workers, in 2003.[93] The authors showed that higher order Volterra kernel slices could be reliably obtained and that they had properties that differ from those of conventional OAEs. Thornton and co-workers have also described normative properties of VSOAEs.[86]

Slaven et al (2003) showed that as the stimulus rate is increased from very low rates, the amplitudes increase, reach a maximum and then decrease with further increase in rate.[93] Poor correlations were found between adjacent slices. In the study by Thornton, Lineton, Baker et al (2006) stimulus rates of 1000 or 1500 clicks/s gave the largest number of 'good' slice waveforms both for the second order, denoted S_2 and third order, denoted S_3. [86] Furthermore, at these stimulus rates S_{21} (2^{nd} order slice 1) and S_{31} (3^{rd} order slice 1) were present in the majority of conditions applied in the study. [86] No significant correlation between the amplitude of the second order slices and that of the first order slice was shown, and the authors suggested that they reflected different aspects of cochlear mechanics. A strong correlation was found between the amplitude of the second order slice and the slope of the growth function of the first order slice, indicating a link between nonlinearity in the temporal and amplitude domains.[86]

1.6.4) Objective 2

Conventional EOAE amplitude differs between ears and sexes; female subjects having responses of greater amplitude than male subjects and right ears larger responses than the left. The question to be answered is whether these differences occur with VSOAEs, whether they vary with the rate and are affected by the presence of SOAEs.

1.6.5) Objective 3

Theoretically all measures of OAE nonlinearity should relate to one another as they are likely to represent a similar mechanism occurring in the cochlea, or indeed have a common origin. Thus the relationship between SOAEs, DPOAEs, CEOAE I/O function and VSOAEs, and their interaction with one another is investigated. The question being 'How do VSOAEs as indicators of nonlinear mechanism relate to other OAE measures of nonlinearity?'.

1.7) Summary of objectives

This thesis addresses three research questions. CEOAEs amplitude differs between sex and side, with females having emissions of greater amplitude than males, and right ears emissions of greater amplitude than left ears. Firstly, as a pre-requisite to clinical use, it is necessary to establish if these sex and side differences occur with the MLSOAE technique, and whether they change with stimulus rate. Secondly, whether these sex/ side differences occur with VSOAEs. Thirdly, the relationship of VSOAEs with other measures of nonlinearity (SOAEs, I/O function of CEOAEs and DPOAEs) was investigated.

CHAPTER 2
METHODS

2.1) Recruitment of subjects

The study took place at Medical Research Council, Institute of Hearing Research at the Royal South Hants hospital, Southampton. Informed written consent was obtained from all participants, who were normally hearing and aged between 18 and 40 years. The subjects were recruited from those answering advertisements placed in the University of Southampton, the hospital and also medical students responding to an email request for subjects of normal hearing. All participants were given an information sheet prior to obtaining their consent (**Appendix 1**) for both series of experiments.

Subjects were required to answer a questionnaire and undergo otoscopy, as detailed below, before the commencement of any audiological tests. The aim of the questionnaire, which can be seen in **Appendix 2,** was to exclude those with any indication of cochlear pathology, ensuring only those with no significant history of ear problems proceeded to the next phase of testing.

The study comprised two parts and included 81 ears for the first series of experiments and 45 ears for the second series of experiments. Recruitment of participants was difficult, perhaps due to the location, length and timing of the testing. The study was a normative parametric study.

2.2) Tympanometry and Audiometry

In the first series of experiments to look at the MLSOAE interaction with sex and ear, eighty-one ears of forty-four normally hearing adult volunteers between the ages of 18 and 40 years were tested. Both ears were tested in thirty-six subjects. Ears were balanced for sex and side, with 20 male left and 21 male right ears, and 20 each of female right and female left ears. In the second series of experiments to investigate the effect of sex, ear and SOAEs on VSOAEs, and the relationship of nonlinear measures with one another 45 ears of 25 subjects were tested. These

comprised 25 female ears and 15 male ears. Both ears were tested in 20 subjects; 14 females and 6 males. For all subjects, the standard clinical tests of otoscopy, pure tone audiometry and tympanometry (Grason-Stadler GSI-33) were performed.

The otoscopic examination was used to check for and ensure the normal status of the tympanic membrane and middle ear. Subjects in whom ear canals were occluded by cerumen did not undergo testing of the affected ear, since Chang *et al.* (1993) demonstrated that cerumen hinders the measurement of OAEs.[95]

The ears included in the study were free from middle ear dysfunction as confirmed by tympanometry pattern type A (Jerger *et al.*, 1974), (MEP between -100 and +50 dPa); with compliance between 0.3 and 1.5 ml.[96] Previous studies have shown the effect of middle ear pressure on TEOAEs, with the equalization of middle ear pressure (obtained by recording TEOAEs at an ear canal pressure equal to the tympanometric peak pressure), resulting in an increase in TEOAE amplitude.[97] A negative middle ear pressure was also shown to affect the stimulus, recorded in the ear canal by the ILO88 system, with a peak seen in the stimulus spectrum, suggesting that middle ear equalization allows for the more optimal presentation of a stimulus.[97] Evoked otoacoustic emissions (EOAEs) are detected most distinctly at the middle ear resonance frequency (where the eardrum vibrates with the largest displacement amplitude, and sound energy is transmitted efficiently into the cochlea), which in normal subjects has been shown to be between 0.8 and 1.5 kHz.[98] They are most detectable in normal subjects whose middle ear mobility is moderate, i.e. the amplitude of the EOAE at the best frequency increases until the degree of middle ear mobility expressed as a value is about 5 dB and decreases thereafter.[98]

A hearing threshold of equal to or less than 20 dB HL was required and confirmed by pure tone audiometry (Kamplex KC 50 Audiometer) via air conduction at all octave intervals between 125 and 8000Hz. The resolution of the clinical audiogram was 5dB.

In addition, the subjects also completed a questionnaire (see **Appendix 2**) as described earlier. The subjects presented no histories of hearing disorders and were free of major pathology. Subjects whose ears were tested on two separate occasions underwent repeat tympanometry and otoscopy to ensure the ear was still normal.

A sound insulated audiological test booth was used in all parts of the experiment, with the subject instructed to sit quietly and as still as possible relaxing in a comfortable, reclining chair. The subject was asked to swallow as infrequently as was comfortable during the recordings.[99] A good fitting probe was applied to the external auditory meatus to provide a good seal for the tympanometry measurements.

The experiments were performed in accordance with the guidelines of the Declaration of Helsinki and approved by Southampton and South West Hants Local Research Ethics Committee (submission numbers 105/01 and 264/03/w).

2.3) Conventional OAE recording methods

Otoacoustic emissions (OAEs) are low level acoustical signals that can be recorded in the outer ear canal. They arise from the outer hair cells (OHCs) of the cochlea, the OHCs are electromotile cells that change their shape elongating and contracting, most of this energy is passed to the inner hair cells. However in doing so they emit their own acoustical signal, and also an imperfection of this mechanism is not all the energy is transmitted to the inner hair cells and therefore, this energy in the form of an acoustical signal passes through the middle ear and can be detected in the outer ear canal by a securely fitting probe. OAEs are measured by presenting a series of very brief acoustic stimuli, clicks, to the ear through the probe that is inserted in the outer third of the ear canal (**Figure 2.0**). The probe contains a loudspeaker that generates clicks and a microphone that measures the resulting OAEs that are produced in the cochlea and are then reflected back through the middle ear into the outer ear canal. The resultant sound that is picked up by the microphone is digitized and processed by specially designed hardware and

software. The very low-level OAEs are differentiated by the software from both the background noise and from the contamination of the evoking clicks.

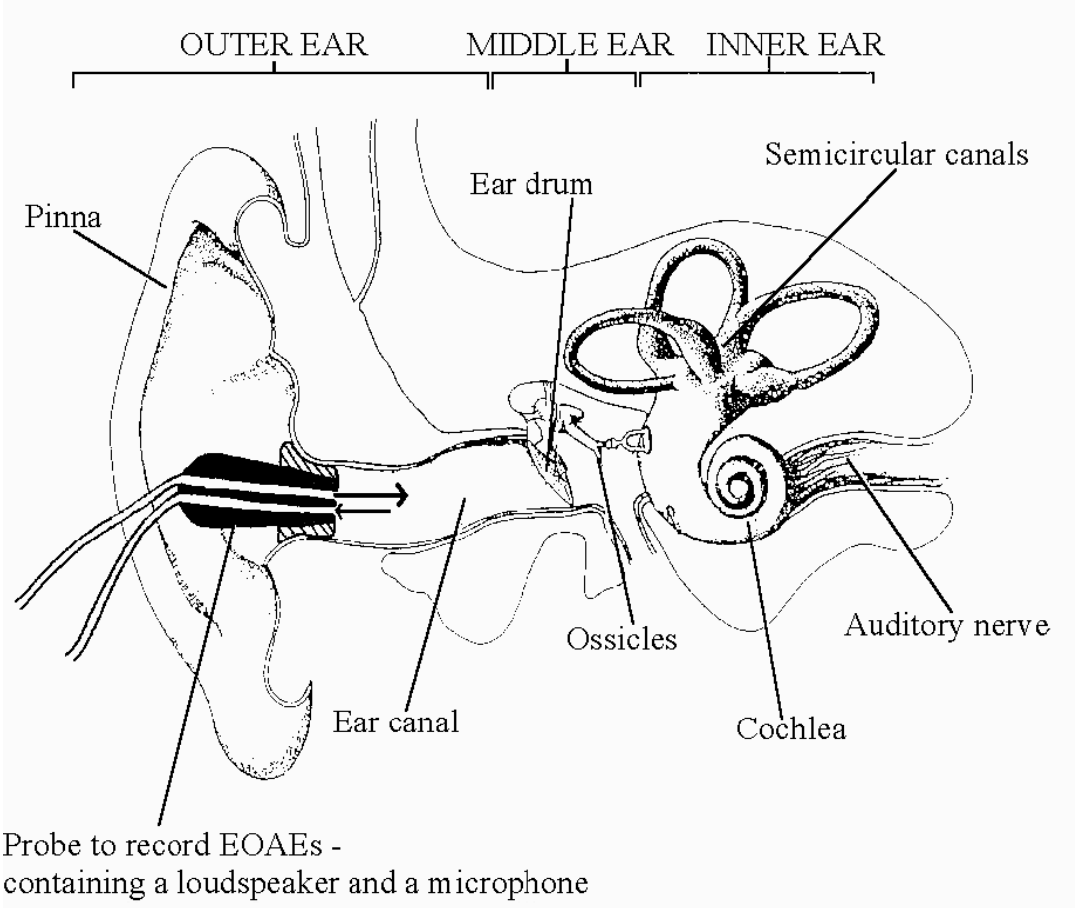


***Figure 2.0**. OAE testing probe securely fitted into external auditory meatus.*

The slope of the Input/Output function of the conventional click evoked otoacoustic emission (CEOAE) can be taken as a measure of nonlinearity; hence the conventional recording method requirement.[100] In current practise conventional OAEs can be recorded using hand held machines with attached probes (i.e. Otodynamics ILO-88).

In this study to obtain as optimum, uncontaminated results as possible, subjects were tested in a sound insulated, sound attenuating audiological test chamber and required to sit as still and as quietly as possible.

Conventional testing was undertaken using an IHR in-house designed system comprising a probe, amplifier and a filter of a Maximum length sequence otoacoustic emission (MLSOAE) measurement system consisting of specific DSP boards in an IBM compatible PC. This system is capable of recording both conventional and MLSOAEs. The probe consisted of a Knowles microphone (box J, probe D, serial number SGS-5D906006) and transmitter embedded in an appropriately fitting plastic ear plug. The equipment was then calibrated before testing on any subjects using a Bruel and Kjaer sound level meter, microphone and 2cc cavity. Using the measuring equipment, the linearity (for the input/output function) of the sound delivery system was verified.

Configuration files were constructed to enable easier and more uniform data storage. For the conventional I/O testing, at a rate of 40 clicks/s 8 files were written for the 4 different stimulus levels one for each of the following; 40, 50, 60, 70 dBpeSPL respectively and then for the second run obtained in reverse order 70, 60, 50, 40 dBpeSPL respectively. Each run was recorded twice at each level, in order to ensure good waveform correlations were obtained and could be calculated when analysing the data. The recording window for the conventional OAEs was 0-17 ms, so that responses could be compared with any residual stimulus artefact expected in the earlier part of the time window, generally taken as the 0-5 ms portion.[101] The presence of an emission at each level was accepted if the correlation coefficient between repeat waveforms for the 6-17 ms, and 9-13 ms portion of the emission was >0.5, the traces were free of spurious artefacts and the waveforms showed the typical characteristics of an OAE, as have been shown to produce good results in previous studies.[102] This portion of the window for which the correlation is calculated is also free from any residual stimulus artefact, as mentioned earlier. The sound levels at which subjects were tested were used as they have been previously shown to yield good results.[100] At all stimulus levels responses were averaged for 25 seconds, equating to a 1000 clicks at rate 40/s. The stimulus level was an unfiltered click of uniform duration. The order in which the levels were presented was counterbalanced across the ears tested.

2.4) MLS Recording

Thornton and colleagues have stated ‘One of the very practical problems of using evoked otoacoustic emissions (EOAEs) to test neonates and young children is that, to obtain a good recording, responses must be averaged over a period of a minute or so; the child or baby must therefore be quiet for that length of time’. ‘To achieve this quiet period can take many more minutes of testing because the equipment will reject sweeps that are contaminated by noise or movement artefacts’.[73] This problem can be approached by reducing the test time. In addition to this if neonates are tested within the first three days of life, then the overall failure rate is unacceptably high. Thornton et al (1993) have shown that with 50% of babies being discharged within the first three days, 36% of normally hearing babies would be expected to fail evoked emission testing using the conventional equipment.[73] In the first three days of life the emission is more than likely to be present but at a smaller amplitude. Therefore a more sensitive recording technique is desirable.[73]

When utilising click EOAEs a number of response epochs must be averaged to improve the signal-to-noise ratio, thus producing a clear waveform. However, the maximum click presentation rate is limited by the window or epoch length; the window normally being of the order of 20 ms and so the maximum stimulation rate is about 50/s.[69] If it were possible to increase the click presentation rate in order to reduce the test period, the responses would overlap each other and the stimulus clicks and corrupted waveforms would result. This problem can now be overcome as EOAEs can be recorded using maximum length sequence (MLS) techniques. The Medical Research Council Institute of Hearing Research at Southampton has developed a new EOAE technique (MLS OAE), which enables stimulus rates of up to 5000 clicks/s to be used (**Figure 2.1**).[73]

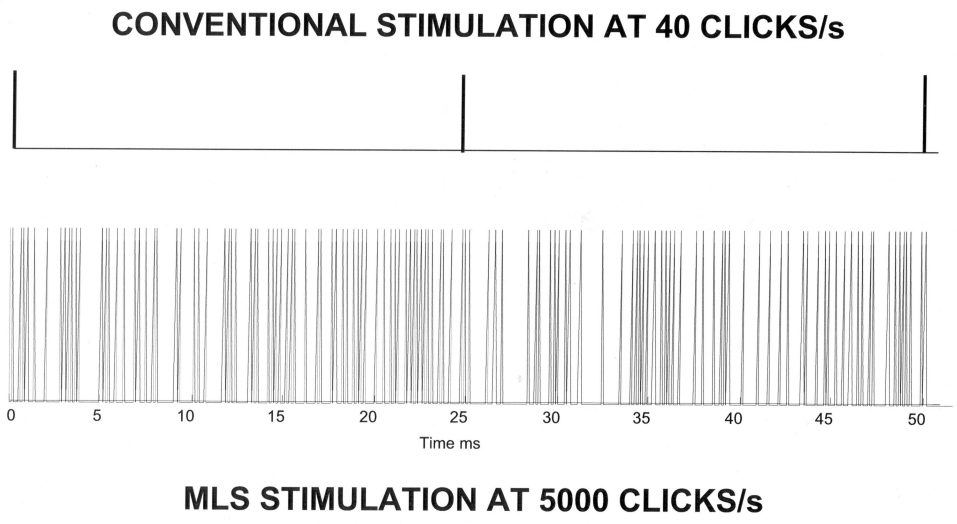


Figure 2.1. *Conventional and MLS stimulation.*

The equipment consists of standard DSP boards and computers. This technique allows considerable reduction in recording time and a greater range of stimulus rates compared with conventional recording. The gain in signal-to-noise ratio obtained by stimulating at rates of up to 5000 clicks/s enables this technique to detect responses that are only 20% of the amplitude of the responses that are detectable by the conventional technique in the same recording time. Previous studies of MLS transient evoked otoacoustic emissions (TEOAEs) in adults have shown that there is a decrease in emission amplitude with increase in stimulus rate.[103, 104]

The data for the study were obtained using the IHR in-house designed MLS system comprising the probe, amplifier and filter of a MLSOAE measurement system.[73] The probe comprised a Knowles microphone and transmitter embedded in a plastic earplug. Every effort was made to ensure the end of the tip was flush with the probe. The probe was placed in the ear canal and checked. MLSOAE's were measured at eight stimulus rates (clicks/s): 40 (conventional), 300, 500, 1000, 2000, 3000, 4282 and 5000/s. These values, and all subsequent references to MLS click rate, represent

the maximum rates occurring within an MLS, that is, the reciprocal of the minimum time between clicks.[69, 105] All MLS emissions were collected using an order 10 MLS and conventional recordings were carried out at 40/s. The preset number of clicks, which depended on the stimulus rate, was calculated so that each run took approximately thirty seconds to acquire. The number of clicks recorded at each MLS and the order are shown in **Table 2.0** below.

Rate (clicks/ second)	**Order**	**Number of MLS clicks**
40	1	1200
300	10	4608
500	10	7680
1000	10	14848
2000	10	30208
3000	10	45056
4282	10	64512
5000	10	75264

Table 2.0. *The approximate number of clicks recorded at each rate.*

These numbers enabled clear MLSOAEs to be obtained whilst keeping the overall session duration no longer than two hours. Two runs were recorded at each stimulus rate and at each of two stimulus levels, 60 and 70 dB dial (the term dB dial refers to the equipment/screen setting). At all rates the analysis window was 5 to 17 ms post stimulus. An Fsp criterion of greater than 3 was obtained at each run to ensure a good emission and the shape of the waveform also monitored for spurious artefacts. The Fsp is a statistical measure that gives an indication of the “quality” of the signal (related to the signal-to-noise ratio), and a value of 3 has been found to give highly repeatable (well correlated) waveforms.[104, 106, 107] The conventional method of calculating the Fsp divides the variance at a single time point in successive raw (unaveraged) responses by the variance of the whole, averaged response.[104] For the MLS it is applied to the reconstructed MLS, and the variance for a single time

point in the reconstructed response used as a numerator and each reconstructed MLS is then added into a summating buffer, allowing the averaged trace variance to be calculated.[104] The rates were presented using a balanced Latin square design (see **Tables 2.1** & **2.2**) and the first ear to be tested was alternated between the left and right. All measurements were made with the subject sitting, relaxed in a sound proof chamber.

The first ear to be tested was alternated between subjects, as a significant effect of test order has been found in the past, with the measured right/left ear difference being enhanced when the right ear is tested first and diminished when the left ear is tested first.[108] In some cases this was not possible as only one ear could be tested.

Representative letter	Rate (click/s)
A	40
B	300
C	500
D	1000
E	2000
F	3000
G	4282
H	5000

Table 2.1. *Latin square key.*

Subjects	Latin Square	Order of Presentation
1, 9, 17, 25, 33, 41,49, 57, 65, 73, 81	A B H C G D F E	**40 300 5000 500 4282 1000 3000 2000**
2, 10, 18, 26, 34, 42, 50, 58, 66, 74	B C A D H E G F	300 500 40 1000 5000 2000 4282 3000
3, 11, 19, 27, 35, 43, 51, 59, 67, 75	C D B E A F H G	**500 1000 300 2000 40 3000 5000 4282**
4, 12, 20, 28, 36, 44, 52, 60, 68, 76	D E C F B G A H	1000 2000 500 3000 300 4282 40 5000
5, 13, 21, 29, 37, 45, 53, 61, 69, 77	E F D G C H B A	**2000 3000 1000 4282 500 5000 300 40**
6, 14, 22, 30, 38, 46, 54, 62, 70, 78	F G E H D A C B	3000 4282 2000 5000 1000 40 500 300
7, 15, 23, 31, 39, 47, 55, 63, 71, 79	G H F A E B D C	**4282 5000 3000 40 2000 300 1000 500**
8, 16, 24, 32, 40, 48, 56, 64, 72, 80	H A G B F C E D	5000 40 4282 300 3000 500 2000 1000

Table 2.2. *Latin Square.*

2.5) VSOAE extraction

As described in **Chapter 1** Volterra kernel analyses of responses obtained from MLS stimulation enables second and higher order nonlinear components to be measured.[73] These kernels provide a more accurate picture than the linear (first order-MLSOAE) response of the actual response of the hearing mechanism, as the hearing system is 'neither linear nor time-invariant'.[73] The Volterra kernels are continuous functions of time, whereas the MLSs are discrete, binary sequences with a maximum resolution in the time domain, corresponding to the minimum time interval between clicks.[87] Therefore, at a stimulus rate of 5000 clicks/s the resolution is 0.2 ms, thus samples of the kernel called slices can be obtained for values of τ (the range over which the integration is carried out) equal to multiples of 0.2ms.[87]

In this study the VSOAE were obtained using the same in-house designed system as was used for obtaining the CEOAE I/O function results. An MLS Order 12 was used when obtaining the VSOAEs. The stimulus rate (click rate) was 800 or 1000 or 1200. Click rates of 800, 1000 and 1200 clicks/s were selected as the highest amplitude responses for the second and third orders of the VK are obtained at around this rate.[100] Averaging was terminated when the predetermined number of clicks had been averaged. The number of clicks was based on earlier findings and was chosen to give a good signal-to-noise ratio at each of the stimulus rates.[100] Twelve MLS reconstructions were recorded; this meaning that each recording (run) was comprised of twelve complete MLSs at each stimulus rate. The click level used was 70 dBpeSPL, as this has previously been shown to produce good results by this group.[100]

The same MLS system which was used to collect the VSOAE and CEOAE I/O function data was calibrated as detailed earlier (**section 2.3**). Probe 'D', serial number SGS-5D906006 and box 'J' were used. To further improve the signal-to-noise ration two repeat runs were performed at each of the stimulus rates. The rate presented first was alternated between subjects. The recording time required to obtain VSOAEs at a

stimulus rate of 1200 clicks/s was 50 seconds and at a stimulus rate of 1000 clicks/s was 60 seconds, and at a stimulus rate of 800 click/s was 65 seconds. The test duration was approximately 6 minutes for all the VSOAEs.

2.6) DPOAE recording

DPOAE testing was undertaken with the subject relaxing in a reclining chair in a sound attenuated chamber. A requirement of subjects included in the study was that they had not been exposed to pure tones of wide band noise at a level of 80 dBHL, at least 3 minutes prior to the DPOAE being measured. This was necessary as Kiss et al. (2001) demonstrated that DPOAE amplitudes changed immediately after pure tone and wide band noise exposures of levels 80 dBHL at most frequencies.[109]

In order to decide on the best frequency range and, F1 and F2 levels, various other studies were reviewed. Lonsbury-Martin (1990) investigated the properties of DPOAEs in normally hearing subjects and detailed testing included the recording of DPOAE grams in 100-Hz steps from 1 to 8 kHz at three primary-tone levels (65, 75, and 85 dBpeSPL).[110] In addition, response-growth or input-output (I/O) functions depicting the relationship of the amplitudes of DPOAEs to primary-tone levels, ranging from 25 to 85 dB SPL in 5-dB steps, were also tested for 11 frequencies distributed at quarter-octave intervals over the identical frequency range.[110] The average DP-gram illustrating the frequency response of these emissions demonstrated a bilobed contour having a low-frequency maximum at approximately 1.5 kHz and a high-frequency peak that plateaued at about 5.5 kHz.[110] The two maximum regions were separated by a minimum around 2.5 kHz.[110] Depending on the frequency region, the average I/O functions exhibited detection "thresholds" at 3 dB above the noise floor at primary levels between 35 and 45 dB sound pressure level.[110] The dynamic range of the emitted response between detection "threshold" and maximum amplitude varied over a 40-dB extent of the stimulus-level dimension.[110] Approximately one third of the ears exhibited irregular DP-grams in which

emitted responses were significantly reduced in restricted regions tested by low, medium, or high frequencies.[110] When the 44 ears were separated into two groups representing more-normal and less-normal responses, the irregular "normal" ears demonstrated increased variability, especially in high-frequency regions.[110] Gorga et al (1993) in their study comparing TEOAEs and DPOAEs in normal and hearing impaired ears measured DPOAEs with F2=1.2*F1, with the lower frequency presented at 65 dB SPL and the higher frequency at a level of 50 dB SPL.[111] The frequency range was 500 to 8000Hz with three points per octave, and it was shown that the DPOAE continued to increase up to about 4000 Hz, beyond which it remained relatively constant or decreased.[111] This study also showed DPOAEs performed comparably with TEOAEs at 2000 Hz.[111] Gorga et al (1999) analysed DPOAE and audiometric data from 1267 ears of 806 subjects.[112] These data were evaluated for three different frequency combinations (2, 3, 4 kHz; 2, 3, 4, 6 kHz; 1.5, 2, 3, 4, 6 kHz).[112] DPOAE data were collected for each of the f2 frequencies listed above, using primary levels (L1/L2) of 65/55 dB SPL and a primary ratio (f2/f1) of 1.22.[112] Sensitivity and specificity were evaluated for signal to noise ratios (SNRs) of 3, 6, and 9 dB.[112] They concluded that it should not be assumed that the use of a priori response criteria, such as SNRs of 3, 6, or 9 dB, where sensitivity did not reach 100%, will identify all ears with hearing loss. In their study of normal and hearing impaired ears Probst et al (1990) used pure tone stimuli at fixed frequency levels of 73dBHL for F1 and 67dbHL for F2 and tested frequencies between 1-6 kHz.[113] The frequencies of the two primaries were chosen so that their geometric mean represented standard audiometric frequencies. Measurements of the emission amplitudes at 2F1-F2 and the adjacent noise floor were achieved by spectral averaging. [113] Another study investigating the source of DPOAEs using suppression experiments and inverse fast Fourier transforms, in which DPOAE data were collected in normally hearing adult subjects in a population aged 18 to 32 years, and hence similar to this study, used the following frequencies: with f2 fixed at 2 or 4 kHz respectively, the frequency ranged from 875-1813 Hz and from 1750-3813 Hz respectively.[114] The level differences were manipulated as part of the

experiments.[114] Recommended settings for DPOAE machines (e.g. SmartDPOAE, intelligent hearing systems) used in practise are shown below, **Table 2.3**).[115]

Frequencies:	Diagnostic: 500-8000 Hz Screening:1200-4000 Hz
Frequencies per octave	Diagnostic: 2 or more Screening: 2
F1/F2 ratio	1.22
Sweep	32 sweeps per frequency maximum for diagnostic
Block size	8 sweeps per block
Level	65 dB SPL Maximum for Level 1 55 dB SPL for Level 2
Passing criteria	65% or higher

Table 2.3. *Recommended setting for DPOAE acquisition, for commercially available units.[115]*

The theory of DPOAE backscattering of waves was also taken into account.[116] This theory can be summarised as follows. The measured distortion product (DP) is thought to have two main components. The travelling wave overlap region acts like a source of DPs sitting on the basilar membrane, radiating both forward and backward DP travelling waves. The backward wave comes straight out where it is measured. The forward wave goes apically till it reaches the characteristic place of the DP (which is always apical of the generation site, if looking at 2f1-f2, rather than 2f2-f1). At the characteristic place, it may well be reflected back. These two components then create an interference pattern in the ear canal (and give rise to ripples or fine structure in the DP-gram). In this study the size of the DP as an index of the degree of nonlinearity was required. However, if a single line was only measured we would not have known if we were at the peak or trough of the interference pattern, i.e. is a DP of large amplitude indicate a greater degree of nonlinearity or simply constructive interference. Over a number of ears this phenomenon would average out, but in order to reduce unnecessary variability in our measure of nonlinear frequency interaction components

(DPOAEs) averaging over frequency for each ear was undertaken, thus providing an estimate of the strength of the DPOAE due to overlap alone, at the centre of the frequency average. It is important to note that the relative contribution from the DP place decreased as the primary level increased towards levels typically used under clinical conditions.[116] Thus less influence would be expected from the DP reflection source under the stimulus conditions in which DPOAEs are measured clinically.[116]

Based on these previous studies' settings, our population, our own experiences and the aim of the experiment to compare DPOAEs to VSOAEs, the pure tone stimuli were presented at fixed levels of 73 dB SPL for F1 and 67 dB SPL for F2. Frequencies of the two primaries have been chosen so that their geometric mean represents the standard audiometric frequencies. For the reasons indicated above and in order to avoid the backscattered wave phenomenon, a sweep between 750 Hz and 4 kHz was obtained, with 32 Hz increments between successive frequencies. The mean value of the DPOAE was then calculated from this. The DPOAE settings used, where ear side was varied accordingly are shown in **Table 2.4**.

Global settings	
Ear	Left
Test type	Sweep
Named setting	dp750-4
Maximum buffers	100
Rejection level (dB)	10
Auto stopping	Enabled
Minimum buffers	10
Minimum SNR (dB)	102
Buffer size	102
Sweep type	Fixed ratio plot by F1
DP to track	2F1-F2
Lower sweep limit (Hz)	750
Upper sweep limit (Hz)	4000
Fixed parameter	1.22
F1 level (dB)	73
F1 phase (degrees)	0
F2 level (dB)	67
F2 phase (degrees)	0
Linear/logarithmic	Linear
Sweep increment (Hz)	32

Table 2.4. *Settings used for DPOAE acquisition in this study.*

Prior to obtaining DPOAE data for subject ears a recalibration of the DPOAE equipment was undertaken. A calibration sequence was conducted in which the outputs as a function of frequency (from frequencies of 256 Hz to 10240 Hz) were evaluated when a constant voltage was applied to the two earphones (A and B). Any corrections necessary were made and the microphone recalibrated again to check no change in the result from earlier. Microphone BK 4144 serial no 704097 was used.

The same in-house designed system used to collect the SOAEs was used to record DPOAEs, but on the DPOAE setting. The microphone preamplifier was set at 40 dB gain.

The subject, who was required to remain still and quiet, was placed in a comfortable, reclining chair in a sound proofed booth. A probe with an appropriate tip, to provide a good seal and reduce any residual external noise was placed in the external ear canal of the ear to be tested. Two DPOAE sweeps were obtained, in order to check the repeatability, each of sweep duration approximately 3 minutes.

2.7) SOAE recording

This was undertaken using an in-house system designed by the Medical Research Council Institute of Hearing Research. The gain was set at 40 dB on the ER-10C DPOAE probe driver preamp. The rejection level for the SOAE was set at ~5% of those counted. A rejection level of 17dBHL was used and the buffer set to 400, and this resulted in a rejection level close to that desired (~5%), hence these settings were used for this study.

The SOAE recording was acquired using a well-fitting ear probe at the start of the experiment. The measurement was then repeated at that time and a further two repeat measurements were made at the end of each recording session. Each response was acquired over 30 seconds. In previous studies subjects with spectral peaks >5 dB above the noise floor level have been omitted as this may affect the I/O function of CEOAEs.[86]

2.8) Statistical methods

2.8.1) Basic Principles

The systematic approach to designing an experiment was employed, as described below. Data was then be collected and analysed by statistical methods in order to provide valid and objective conclusions. The study was of a normative parametric design. There are three basic principles of experimental design that need to be taken into consideration. These are Randomisation, Blocking and Replication.

Randomisation is a very important construct and is significant in the use of statistical methods in experimental design. Randomisation implies that both the allocation of the experimental unit and to whom the experimental unit is applied is performed randomly. As such, this study was not randomised as those subjects of normal hearing were selected.

Blocking is a technique used to increase the precision of an experiment. A block is a proportion of the experimental unit that should be more homogeneous. Blocking involves making comparisons among the condition of interest in the experiment within each block. These experiments were of repeated measures design, therefore blocks were not used.

Replication refers to a repetition of the basic experiment. Replication has two important properties. The first is that it allows the investigator to obtain estimates of the experimental error. This estimate of error becomes a basic unit of measurement for determining whether observed differences in the data are really statistically different. Secondly, if the sample mean is used to estimate the effects of a factor in the experiment, then replication aids the investigator to obtain more precise estimates of the effect. Thus several runs for each measure were performed in these experiments.

2.8.2) Experiments to compare several effects

2.8.2.1) Distribution of results

Histograms were used to assess the size and distribution of the data sets and also highlight any skewed distributions and outlying data points.[117] If the bars on the histogram follow a similar pattern to the bell-shaped curve it is assumed the results are obtained from a normally distributed population.[117] Scatter plots were also used to compare the distributions of groups on a single variable and also for single variables.[117] If there was any concern about the distribution of the data

following visual inspection a Kolmogorov-Smirnov test was used to statistically test for normal distribution.[117] This test takes the observed cumulative distribution of scores and compares them to the theoretical cumulative distribution for a normally distributed population.[117]

2.8.2.2) T-tests

T-tests can only be undertaken if there are no other confounding factors and the groups are chosen so that the only difference between them is the one being investigated.[117] The T-test is a parametric test thus the samples must be randomly and independently chosen from the population.[117] The data must come from normally distributed populations and the data from the two groups needs to come from populations with equal variances, although results may still be used if this is not the case.[117]

The independent samples T-test is utilised for unrelated samples for example when comparing female responses with male responses, or right ears of one sample with left ears from a different, independent sample.[117] The Levene's test for equality of variances indicates the result values to be used. If the test statistic F is significant, the two variances differ significantly (which should not occur with a parametric test as equal variances are assumed), and the bottom values obtained (equal variances not assumed) should be used.[117]

The paired samples T-test is used for related samples usually with the same participants in each group, for example comparing female right and left ear responses or male right and left ears responses.[117]

2.8.2.3) The general linear model

The basis of a wide range of statistical tests is general linear modelling, especially ANOVA and regression.[117] By using this method there is greater flexibility in analysis, with as many groups and variables as required, yet the same basic model structure underlies these analyses.[117] These experiments involved more than two effects, for example looking at sex, ear differences, and stimulus rate in the first series of experiments, and the interaction of various measures of nonlinearity in latter experiments. The appropriate procedure for testing the equality of several means is the analysis of variance. It is probably the most useful technique in the field of statistical inference.

Univariate analysis can be used when there is a single dependant variable, and all independent measures are of an independent measures design.[117] Multivariate analysis of variance is used when there is more than one dependant variable and independent variables.[117] Repeated measures analysis of variance or MANOVA is selected when one or more of the independent variables is repeated measures. When there is a single dependant variable, and all independent measures are of an independent measures design.[117] In the MANOVA the within subject effects are the stimulus rate, slice and order. The Huynh-Feldt test result (F, significance value) was taken for the significance for the within subjects effects. The between subject effects are the presence of SOAEs, gender (sex) and ear side. The Wilks Lambda test result (F, significance value) was used for the between subject effects. If more than two comparisons are made a Bonferroni correction is made. The pairwise comparisons of a number of means results in an increase in the risk of a Type I error, this needs to be controlled for and a Bonferroni correction corrects for this. When there is a single dependant variable, and all independent measures are of an independent measures design.[117]

2.8.2.4) Summary of statistical tests used in experiments

In the experiments to test for normality distribution curves and the Kolmogorov-Smirnov test were used. In the statistical analyses of these results, independent and paired T-tests, together with the Bonferroni correction, analyses of variance (including general linear model), linear correlation and regression techniques were all used. The ANOVA was used to test the significance of the regression model.

In statistics the term ‘correlation’ indicates the strength and direction of a linear relationship between two random variables.[118] In general statistical usage, *correlation* refers to the departure of two variables from independence.[118] There are a number of different coefficients used for different situations.[118] The best known is Pearson’s correlation coefficient, which is obtained by dividing the covariance of the two variables by the product of their standard deviations.[118]

CHAPTER 3

RESULTS 1: EFFECT OF SEX AND SIDE ON MLSOAES

3.1) Introduction

Conventional EOAE amplitude differs between ears and sexes; female subjects having responses of greater amplitude than male subjects and right ears larger responses than the left. As a pre-requisite to clinical use it is necessary to establish if these differences occur with the MLSOAE technique and whether they change with stimulus rate.

3.2) Design of study and protocol

This has been detailed in Chapter 2. To recap, Eighty-one ears of forty-four normally hearing adult volunteers between the ages of 18 and 40 years were tested during the study. Both ears were tested in thirty-six subjects. Ears were balanced for sex and side, with 20 male left and 21 male right ears, and 20 each of female right and female left ears. For all subjects, the standard clinical tests of otoscopy, pure tone audiometry and tympanometry (Grason-Stadler GSI-33) were performed and required to be within normal limits. The otoscopic examination was used to check for and ensure the normal status of the tympanic membrane and middle ear. The data for the study were obtained using the IHR in- house designed MLS system comprising the probe, amplifier and filter of a MLSOAE measurement system.[73] The probe comprised a Knowles microphone and transmitter embedded in a plastic earplug, and was placed in the ear canal and checked for a secure fit. MLS OAE's were measured at eight stimulus rates (clicks/s): 40 (conventional), 300, 500, 1000, 2000, 3000, 4282 and 5000/s. These values, and all subsequent references to MLS click rate, represent the maximum rates occurring within an MLS, that is, the reciprocal of the minimum time between clicks.[69, 77] All MLS emissions were collected using an order 10 MLS and conventional recordings were carried out at 40/s. The preset number of clicks, which depended on the stimulus rate, was calculated so that each run took approximately thirty seconds to acquire. The number of clicks recorded at each MLS and the order are shown in **Table 3.0** below.

Rate (clicks/ second)	Order	Number of MLS clicks
40	1	1200
300	10	4608
500	10	7680
1000	10	14848
2000	10	30208
3000	10	45056
4282	10	64512
5000	10	75264

Table 3.0. *The approximate number of clicks recorded at each rate.*

Two runs were recorded at each stimulus rate and at each of two stimulus levels, 60 and 70 dB dial. At all rates the analysis window was 5 to 17 ms post stimulus. An Fsp criterion of greater than 3 was obtained at each run to ensure a good emission and the shape of the waveform also monitored for spurious artefacts. The rates were presented using a balanced Latin square design and the first ear to be tested was alternated between the left and right. All measurements were made with the subject sitting, relaxed in a soundproof chamber.

3.3) Analysis procedure

The MLSOAE waveforms were all inspected for artefacts, and a 5- 17 ms window of the waveform was analysed, using an in-house analysis package written in MATLAB, in which the calculation of RMS amplitude values was carried out. Data were then imported into the SPSS package. The cross correlation between the two runs recorded at each click rate for the waveforms was calculated and waveforms were selected with a correlation greater than or equal to 0.5. The correlations calculated were based on the whole waveform (5 to 17 ms), and for the waveforms between 9 and 13 ms. The 9-13 ms time window was used, both here and in prior

studies, because it contains the most prominent portion of TEOAEs in normally hearing adults , and falls beyond the influence of any stimulus artefacts, providing a genuine uncontaminated response.[14] **Figure 3.0** shows an example of an MLSOAE obtained at the different stimulus rates. One male right ear was excluded due to poor correlations between repeat waveforms obtained and one male subject was excluded due to the amplitude of his MLSOAEs being so variable.

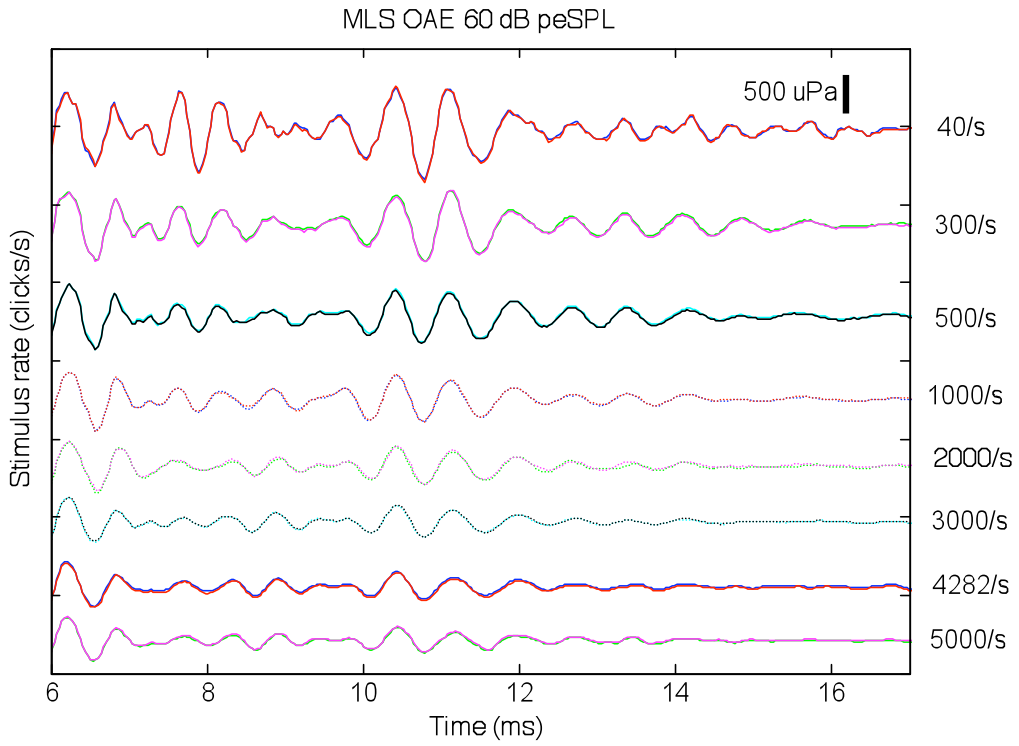


Figure 3.0. *MLSOAE from subject 1, female right ear obtained at 60dB for all 8 rates tested. As the stimulus rate increases the amplitude of the response can be seen to decrease. Also the most prominent part of the response can be seen to occur in the 9-13 ms time interval.*

3.4) Results

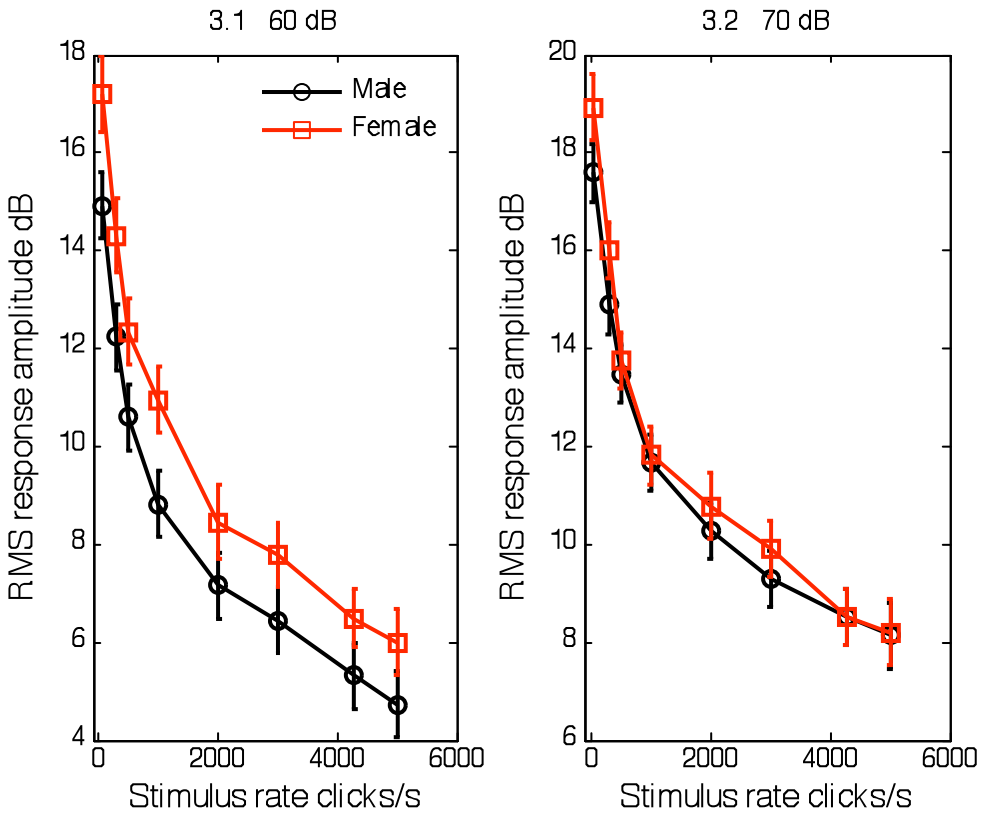
3.4.1) MLSOAE variation with rate

For all subjects, the rms amplitude is expressed in dB re. 20 μPa, calculated for the 9-13 ms window decreased with increasing MLS rate as shown in **Figures 3.1, 3.2, 3.3 and 3.4**, as was expected and has been shown in prior studies.[105] This initial reduction of amplitude with rate reaches a plateau by 1000 to 2000 clicks/s.

An analysis of variance showed that there were statistically significant effects of stimulus rate ($p < 0.0005$) and level ($p < 0.0005$). To examine the effects of rate in more detail the lowest and highest rates were tested to see if the difference between them was statistically significant. The difference between them was shown to be statistically significant.

3.4.2) MLSOAE variation with sex

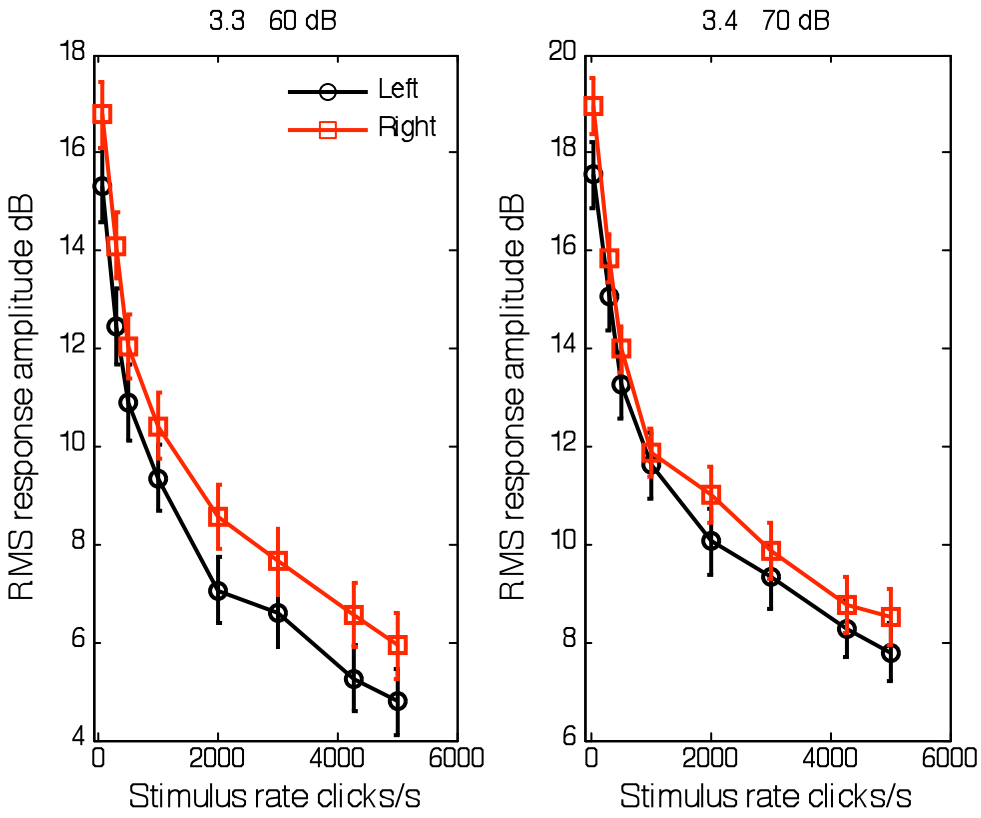
An independent t-test analysis with both levels combined, showed that significantly larger amplitude MLSOAEs were obtained from female ears compared with male ears ($p = 0.005$) at rate 40 clicks/s for the 9- 13 ms window, as shown in **Figures 3.1 and 3.2**. However, at 5000 clicks/s females were not shown to have significantly larger amplitude MLSOAEs than males ($p > 0.05$).



Figures 3.1 and 3.2. *Male versus female, averaged over ears at stimulus levels 60 and 70 dBpeSPL, for the 9-13 ms time window. The results are displayed for 40 male and 40 female ears respectively. The RMS amplitude*

3.4.3) MLSOAE variation with side

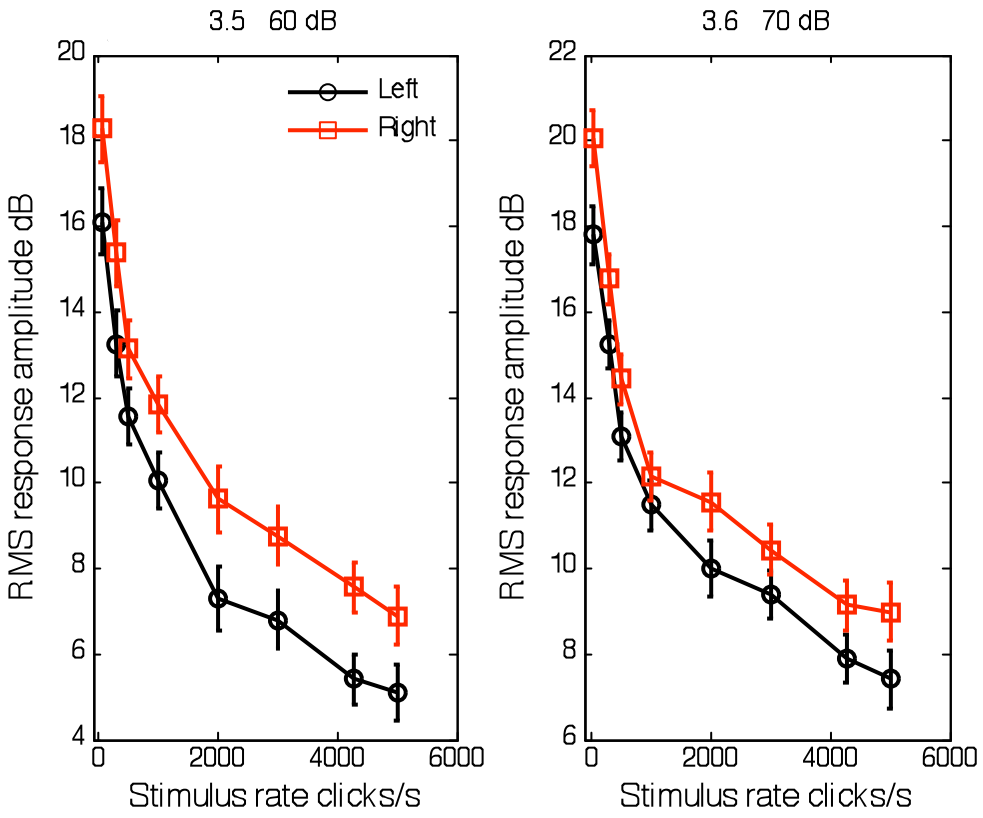
At both stimulus levels right ears were shown to have larger emissions than left ears as shown in **Figures 3.3 and 3.4**.



Figures 3.3 and 3.4. *Right versus left, averaged over sexes at 60 and 70 dBpeSPL, for the 9-13 ms time window. The results are displayed for 40 right ears and 40 left ears respectively. The RMS amplitude is in dB SPL or dB (re 20 μPa).*

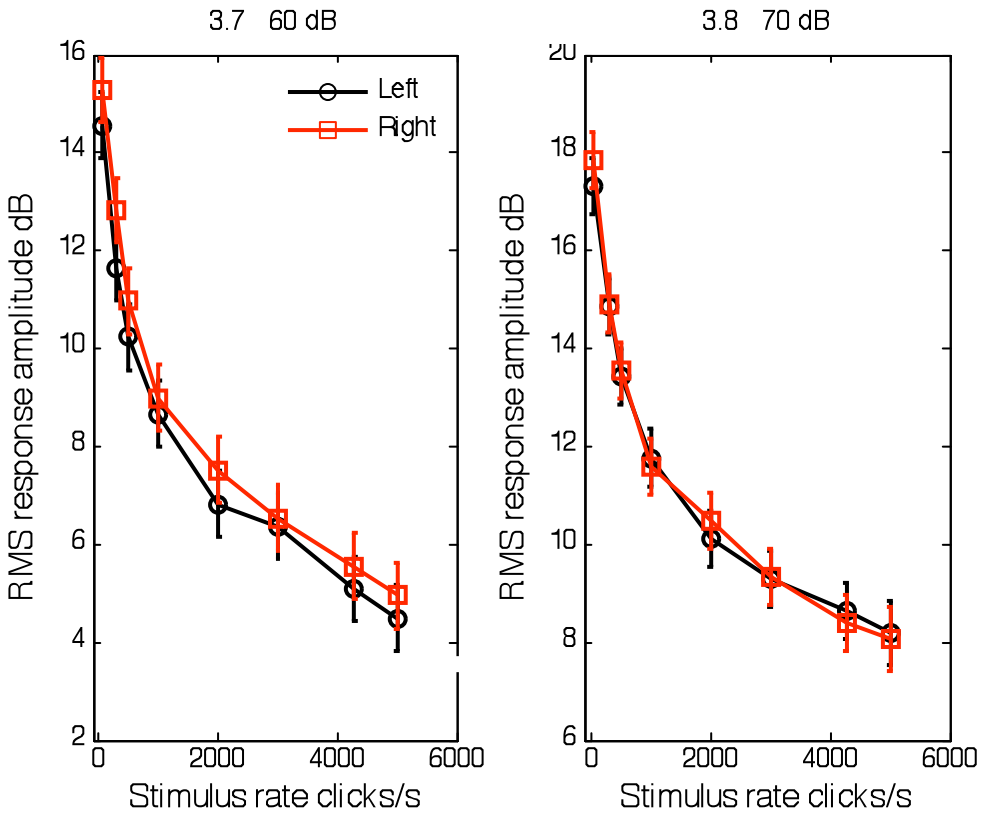
3.4.4) MLSOAE variation with sex and side

Paired samples t-test analysis showed females to emit MLSOAEs of significantly larger amplitude from their right ears than left ears ($p < 0.001$) at rate 40 clicks/s combining both levels. Combining both levels at rate 5000 clicks/s female right ears were also shown to have significantly larger amplitude MLSOAEs than female left ears $p < 0.02$. The female right-left ear asymmetry is shown in **Figures 3.5 and 3.6** at the two different stimulus levels.



Figures 3.5 and 3.6. *Females only, right versus left, at 60 and 70 dB, for the 9-13 ms time window. The results are displayed for 18 female right ears and 18 female left ears respectively. The RMS amplitude is in dB SPL or dB (re 20 μPa).*

In male subjects in whom both ears were tested, paired t-tests combining both levels showed no significant difference in the MLSOAE amplitude obtained between right and left ears; p> 0.05 at rate 40 clicks/s and p>0.05 at 5000 clicks /s . The male right-left ear asymmetry is shown in **Figures 3.7 and 3.8** at the two different stimulus levels.



Figures 3.7 and 3.8. *Males only, right versus left, at 60 and 70 dB, for the 913 ms time window. The results are displayed for 18 male right ears and 18 male left ears respectively. The RMS amplitude is in dB SPL or dB (re 20μPa).*

3.5) Discussion

3.5.1) MLSOAE differences with rate

This analysis provides further normative data on MLSOAEs. As seen in prior studies (Thornton, 1994; Hine and Thornton 1997) with fewer subjects and as expected a rate effect was seen, showing that an increase in the rate of stimulus presentation from 40 clicks/s to 5000 clicks/s resulted in a decrease in the amplitude of the MLSOAE as shown in **Figures 3.1 to 3.8**. Also, as expected, the amplitude of the waves decreased sharply initially and then reached a plateau.

3.5.2) MLSOAE variation with sex

The results have shown that females have MLSOAEs of greater amplitude than males and this result is in agreement with studies undertaken comparing the amplitude of TEOAE emissions between the sexes. The size of the male-female difference is similar for conventional and MLSOAEs, the difference found in this current study being in the region of 1 dB.[54, 119-121] Many theories have been put forward and studies undertaken into why sex differences should occur some of these are reviewed below.

The development of cochlear active mechanisms in humans differs between the sexes. The organ of Corti contains hair cells which act as transducers of the auditory system. The inner hair cells (IHCs) which are less numerous are thought to be the primary sensory receptors of this system, and the outer hair cells (OHCs) which are more numerous appear to subserve a facilitatory role, linked to cochlear active mechanisms (CAMs). Thus the normal functioning of the cochlea involves both active and passive mechanisms and in humans the IHCs begin to function at 25-27 weeks gestation as demonstrated by brainstem auditory evoked potentials.[122] EOAEs are believed to reflect cochlear micromechanical events attributable specifically to OHCs, presumably reflecting electromotile responses to sound stimulation.[123] Characteristics of EOAEs have been shown to change significantly as a function of frequency and gender with increasing conceptual age.[122] In the study by Morlet *et al.* (1996) it was suggested that intersex differences may be due to differences in the OHC populations, such as a higher OHC count in females as shown by Wright *et al.* (1987).[122, 124] Studies undertaken in non-human primates have assumed a relationship between irregularities of stereocilia of the OHCs, impedance of the basilar membrane and SOAE expression.[125] Sex differences are manifest in the auditory system and emerge early in development and may be a congenital phenomena, giving rise to a fundamental difference in the bilateral organisation of the auditory system. Other asymmetries between the sexes, observed at the cochlear level, include a higher prevalence of spontaneous OAEs in female adults, as

mentioned earlier. These differences have been noted in young infants and preterm neonates. The cochlear length is also significantly greater in males, by about 15%, than in females and as the cochlea reaches adult size at around midterm, it is assumed this difference in length persists.[122, 126, 127] A theory is that the shorter cochlea may result in faster response time and better synchronisation of the neural pathways.[128] The study by Morlet *et al.* (1996) also postulated the possibility of some mechanism of regulation of OHC production and/or degeneration during maturation, which in turn differs between sexes, according to cochlear length.[122] With regard to SOAEs, Martin *et al.* (1990) proposed that the smaller volume of the female outer auditory canal amplified the low level SOAE thus making them easier to detect.[129]

3.5.3) MLS OAE variation with side

The amplitude of the MLS OAE from female right ears was greater than that from female left ears. This difference has previously been demonstrated in studies using TEOAEs. The right/left difference in prior studies has ranged from approximately 1 dB to 2-4 dB. Aidan *et al.* (1997) found the mean right-left ear difference in a population of neonates was 1.35 dB and Kei *et al.* (1997) in population of infants found it to be ~1 B and Moulin and Collet *et al.* (1993) found a value of 2-4 dB.[54, 120, 130] This mean right left ear difference was obtained when recording TEOAEs from 270 ears from 135 normally hearing adults; of whom 63 were males aged between 19 and 36 years and 72 were females aged between 18 and 40 years). Thus MLSOAEs show the same ear asymmetry effect as conventional TEOAEs, but can be obtained over a shorter time period.

Although the right/left ear difference was shown to be significant in females tested this was not true for male subjects. As mentioned earlier, prior studies have demonstrated the right ear to emit TEOAEs of greater amplitude than left ears. This study may not have shown this ear asymmetry in the amplitude of the MLSOAEs due to several reasons. The sample size may have been too small, and subsequent power

calculations have shown that if a 1 dB difference is expected than the subject number was indeed too small for this difference to be deemed significant. However, another possibility is that no significant right/left male ear asymmetry occurs with MLSOAEs although this is unlikely to be the case. This would be unlikely as it has been shown in earlier studies that male right ears have larger amplitude OAE emissions than male left ears; for example Newmark *et al.* (1997) showed interaural differences were more pronounced in male than female subjects and right ear responses were larger in amplitude at nearly all test frequencies.[51] The male interaural difference was of the order 1-2 dB. Kei *at al.* (1997) who measured TEOAEs in infants also demonstrated a significant ear asymmetry (response level left ear mean 18.74dB, right ear 19.73dB).[54] In support of the theory that a right/left ear asymmetry that is significant does not occur are several other experimental findings. Cassidy *et al.* (2001) found in their study on TEOAES of 350 subjects, of which 170 were males and 180 were females, the subjects being aged between 38- 42 weeks gestation and full term newborns in the first 48 hours of life, that there was no significant difference due to ear.[55] Right ear (M= 12.37 dB) and left ear (M= 12.88 dB) responses were statistically similar. [55] In agreement with this finding Ferguson *et al.* (2000) on TEOAE recording from 688 ears of a group of 345 adults aged 18-25 years of whom 190 were females and 158 males, found no significant left/right ear difference in emission characteristics and this population sample is of a comparable age to the population tested in the present study.[131]

The effect of the order in which right/left ears are tested is also of great significance. Following the collection of the above data a study from this laboratory showed a significant of test order.[108] The results were obtained from a large population of neonates (21 273). If the right ear was tested first the measured right/left ear difference was 1.5 dB, and if the left ear was tested first, the measured right/left ear difference was about 0.5dB. [108] Indeed following this finding the data collected here was reviewed, and it was noted that for paired female ears the right ear was tested first in 7 cases and the left ear tested first in 11 cases. For paired male ears the right ear was tested first in 8 cases and the left ear tested first in 10 cases. Thus, as the

left ear was tested first more frequently in the case of male subjects, this may account for the lack of a significant right/left difference being found. This effect not only serves to explain discrepancies in this part of the study but also highlights the minimal right /left asymmetry obtained in this large sample of neonates. However, changes in the auditory system occur with age, and this must be taken into consideration with the population studied. As a result of these findings in subsequent experiments the order of testing has been alternated between right and left ears.

There have been several studies investigating why these ear differences occur. The efferent innervation that terminates in the cochlea consists of two components, the medial and lateral olivocochlear systems (the MOC and LOC respectively). Most of the neurons of the MOC synapse directly onto the OHCs.[132] McFadden's hypothesis is that the strength of the efferent influence on the right ear is less than that on the left ear, and less in females than in males.[132] This hypothesis was tested by assessing medial olivocochlear (MOC) activity in the left and right ears, the MOC activity being assessed non-invasively through the contralateral attenuation of EOAEs.[47] The results showed the MOC system to be more functional in the right ear than the left ear, for all the tested population, the same tendency being found among females and males. No significant sex differences occurred in the medial efferent lateralisation, and the results indicated a peripheral auditory lateralisation in medial efferent fibre functioning. Khalfa and Collett (1996) also found a significantly greater right side activity of the MOC system in young right-handed individuals.[47] McGlone (1980) concluded that the brains of right-handed males are more asymmetric than those of right-handed females, in adults and in parallel with hearing asymmetry.[132, 133] This right ear advantage is less marked in left-handed subjects.[134] However, in this current study of MLSOAEs no significant ear side asymmetry was found. Anatomic hemispheric asymmetry has been demonstrated in adults and foetuses; the planum temporale is larger in the left hemisphere of 54% of foetal brains, larger in the right hemisphere of 18% and symmetric in 28%.[135] Another suggestion is that the medial efferent system may initiate or regulate a slow contraction of the OHCs and thus regulate EAOE amplitude.[47] Khalfa *et al.*,(1998)

suggested that a change in stimulus intensity to the right ear did not correspond to a great change in OHC motility, as compared to the left ear and that the right ear cochlear active mechanisms responded less to a slight change in acoustic stimulus intensity than the left ear.[136]

Neuropsychological studies have established that the left superior and middle temporal gyrus are the brain regions involved in language perception.[137] Right-left asymmetry and a difference in degree of lateralisation between males and females have been noted at the central level for language recognition and for sound perception: in particular, the mean amplitude of wave III (auditory brainstem response) is larger when the right rather than the left ear is stimulated.[138] The auditory brainstem wave V component has been shown to be smaller and more delayed in males, while the cochlear summating potential was found to be larger in amplitude and shorter in latency in right versus left ears.[139, 140]

CHAPTER 4

RESULTS 2: THE EFFECT OF SEX, SIDE AND SOAES ON VSOAES

4.1) Introduction

There is evidence for a sex difference in the auditory periphery, as well as the existence of a peripheral lateralisation of the auditory system.[54, 119] Conventional evoked otoacoustic emissions amplitude differs between the sexes and ears; female subjects having responses of greater amplitude than male subjects, and right ears producing larger responses than left ears. In the preceding chapter this was demonstrated to also be the case for MLSOAEs, although the difference between male right and left ears was found not to be significant. The effect of sex and side, and the effect of SOAEs on VSOAEs, was studied in the following series of experiments to see if the effect noted with conventional and MLSOAEs, primarily females having larger emissions than males and right ears having larger emissions than left ears, also applied to VSOAEs. The effect of the presence of SOAEs on VSOAEs and its interaction with sex and side was also studied.

4.2) Design of study and protocol

The study was undertaken at the MRC Institute of Hearing Research. Informed written consent was obtained from all participants who were normally hearing and aged between 18 and 40 years. 45 ears, 15 male ears and 30 female ears of 25 normally hearing adults were tested. Both ears were tested in 20 subjects; 14 females and 6 males.

This was a normative parametric study. All subjects were required to answer a questionnaire and undergo otoscopy. Subjects who suffered from a cold in the previous week were excluded, as were those with any indication of an ear problem as suggested by the questionnaire. Otoscopy ruled out those whose ear drums had an abnormal appearance or were entirely obscured by wax. Tympanometry was undertaken with a Grason-Stadler GSI-33 machine and a Jerger classification Type A tympanogram (MEP between -100 and 50 dPa); with compliance between 0.3 and 1.5 ml was required. Tympanometry was used as an adjunct to examination and

questionnaire, to exclude those subjects with suspected middle ear abnormalities. Audiometric testing was used to ensure normal hearing status. Audiological testing was performed at 1 kHz, 2 kHz, 4kHz, 8kHz, 250Hz and 500Hz. Normal hearing was taken as hearing thresholds of 20 dB HL or better at octave frequencies between 250 and 8000Hz. Each ear was tested at a single session. Tympanometry and otoscopy were repeated on the other ear to be tested to ensure its normality. Once again if the subject had, or had recently had, an upper respiratory tract infection they were excluded.

After ensuring the subject was of normal hearing status, each subject underwent the routine outlined below:

1. SOAE testing
2. DPOAE testing
3. Repeat SOAE testing
4. Volterra Slice OAE (VSOAE) testing
5. I/O testing

A more detailed description of the methods is provided in chapter 2. For all the tests, subjects were tested in a sound proofed booth, relaxing in a reclining chair.

SOAE measurement was undertaken using a system custom built by the Institute of Sound and Vibration Research, Southampton. Four hundred sweeps were obtained, with rejection set at approximately 5%. The SOAE recordings were obtained using a well fitting ear probe at the start of the experiment, and repeat runs were recorded. A repeat measurement of the SOAE was obtained at the end of the experiment, and two runs were undertaken once again.

VSOAES were measured using an in house designed system. Recalibration of the equipment was undertaken prior to the commencement of this series of experiments. Sound level and clicks calibration was undertaken using the Bruel and Kjaer sound level meter and a 2 cc cavity, ensuring a linear response for the stimulus level and preset stimulus parameters were used in each case (configuration files). A Stimulus

level of 70 dBpeSPL was used, as subjects included were of normal hearing, and good amplitude responses have been obtained at this level in prior experiments. Stimulus rates of 800, 1000 and 1200 clicks/s were used as the highest amplitude responses for slice two of the VK are obtained at around this level.[86] Averaging was terminated when the required number of traces had been accepted. Two runs at each rate were recorded for the VSOAEs. The rate presented first was alternated between subjects.

4.3) Analysis procedure

The SOAE files, four for each subject were all examined for artefacts. They were then imported into Microsoft excel. The average magnitude of the spontaneous otoacoustic emission for the four runs at each frequency was calculated as described. A graph was created to demonstrate the variation of the average magnitude of the response of the spontaneous otoacoustic emission on the y axis with the frequency on the x axis as shown below (**Figure 4.0**).

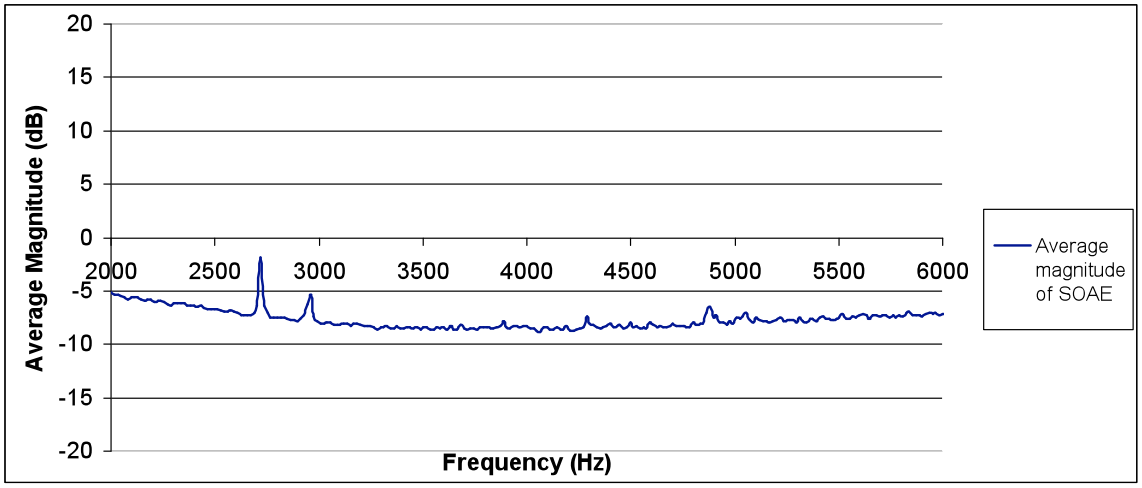


Figure 4.0. *The SOAE trace for subject 2, a female's, right ear, showing SOAEs at approx 2700 Hz and 2950 Hz.*

The average magnitude of the spontaneous emission was then calculated from the base to the tip of the peak. If there was more than one emission this was recorded.

Emissions of magnitude ~3dB or greater were recorded as valid responses. If no emission was present this was also recorded. The results for each subject were then imported into an SPSS file for data analysis.

In order to analyse the VSOAEs several computer programmes were used. The VSOAE waveforms were first deconvolved from the MLS using a programme written in Matlab by Professor ARD Thornton. Statistical analysis was then performed using SPSS and Excel. The individual VSOAEs were deconvolved from the raw responses to MLS. The 1st order slice is the MLSOAE. The CEOAE was obtained using the same MLS system as the VSOAEs, except that the stimulus rate used was the conventional rate of 40 clicks/s. The CEOAE was recorded for four different stimulus levels; 40, 50, 60 and 70 dBpeSPL respectively. Therefore, the CEOAE was analysed in the same way as for the MLSOAEs in **Chapter 3**. The waveforms for the CEOAEs and VSOAEs of every ear were visually inspected to check the waveform lengths, where they started and the waveform correlation. An acceptable correlation was >0.5.

The stimulus artefact is linear and therefore appears on the waveforms of the MLSOAEs. It is much larger than the CEOAEs and MLSOAEs and so the artefact of the original click hides any response for the first few milliseconds. The waveforms for different individuals all start at slightly different times but previous research has shown that population-based time windows provide similar data to subject based time windows and are easier to analyse.[141] For the reasons above the time windows used for the CEOAE/ MLSOAE (first order) were 6-9ms, 9-13ms, 13-17ms and 6-17ms. These have been used in past publications.[100] The stimulus artefact is not present in the VSOAE. The waveforms are shorter and tend to occur earlier, so that different time frames could be compared, 2-6ms, 6-10ms, 2-8ms, 8-14ms, and 2-14ms were chosen.

The root mean squared (RMS) amplitudes of the waveforms were calculated for each response in microPascals. The RMS amplitudes in the different time windows were

calculated and these values were converted from microPascals into decibels (dB re 20 μPa).

RMS in decibels =20*log10(RMS in micropascals/20)

The slices that demonstrated a good response were analysed. The cross correlations between the slices were calculated for the 2-6 ms time window; the physiological signal has been shown to be strongest for the 2-8 ms time interval and this region is free from stimulus artefacts.[80] Furthermore, the 2-8 ms region has been used when analyzing the second and third order responses in prior studies, as it possesses the greatest energy of the response, and resulted in a sufficient number of good quality responses.[100] On preliminary analysis the 2-6 ms time window showed more valid responses compared with the 6-10 ms time window in most cases.

The distribution of the responses for the specific entity being analysed were checked for normality using distribution curves, and following this the one sample Kolmogorov-Smirnov test if there was any uncertainty using the former method. As the use of multiple statistical tests may result in significant results by chance, in the case of independent samples t-tests and paired samples t-tests the Bonferroni correction was applied. The Bonferroni correction for independent samples t-tests was:

p= 0.05/ 12= 0.004
where 12 is the number of independent samples t-tests undertaken in this series of experiments

The Bonferroni correction for paired samples t-tests was

p= 0.05/ 14= 0.004
where 14 is the number of paired samples t-tests undertaken in this series of experiments

The data were analysed using Matlab, SPSS and Microsoft Excel.

4.4) Results

4.4.1) The effect of SOAES on CEOAEs and VSOAEs

Spontaneous otoacoustic emissions were found in the majority of ears, (30 out of the 45 ears, 66.7% of ears tested). They were present in 83.3% (25/30) of female ears tested and in 33.3% (5/15) of male ears tested (**Figure 4.1**)

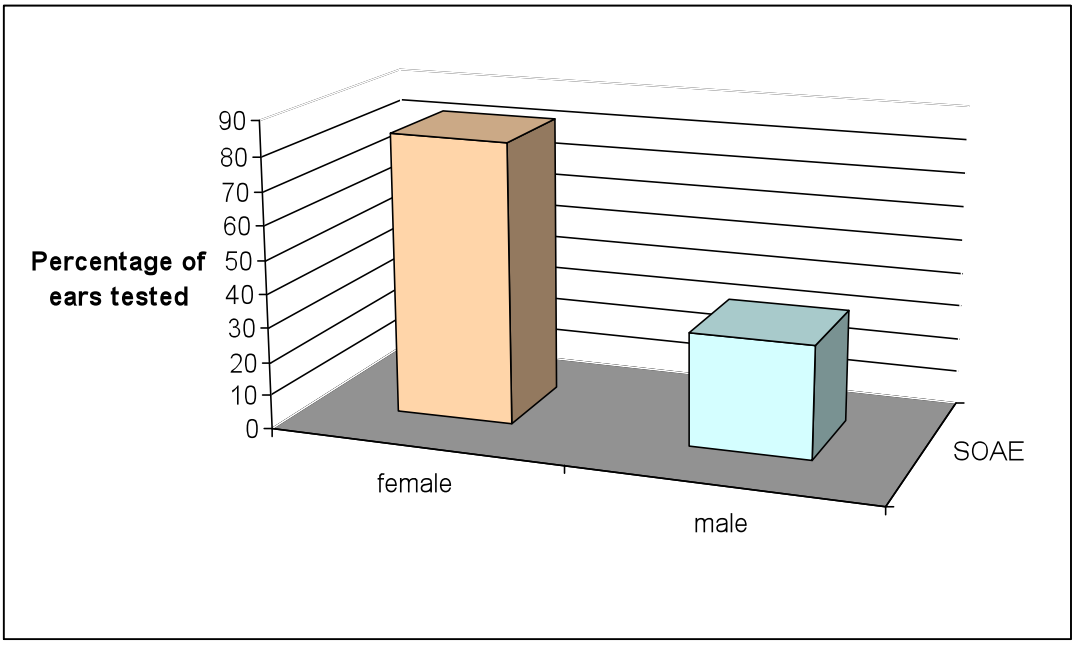


Figure 4.1 *The prevalence of SOAEs.*

As the percentage of females with SOAEs was greater, in agreement with previous findings, and also as less men were tested, this confounds the analysis, hence the need to distinguish between the effect of SOAEs and the effect of sex. **Table 4.0** below shows the SOAE status for females, males, right and left ears.

SOAE	Number of Female Right Ears	Number of Female Left Ears	Number of Male Right Ears	Number of Male Left Ears	Total
Absent	1	4	6	4	15
Present	14	11	2	3	30
Total	15	15	8	7	45

Table 4.0. *Number of ears in which SOAEs absent or present; for female right, female left, male right and male left ears respectively.*

The effect of SOAEs on the CEOAEs, obtained at 40 clicks /s at the different levels was tested. The number of valid responses (those with a correlation >0.5) in subjects with and without SOAEs were compared for the CEOAEs, at the levels tested and is shown in **Figure 4.2** below.

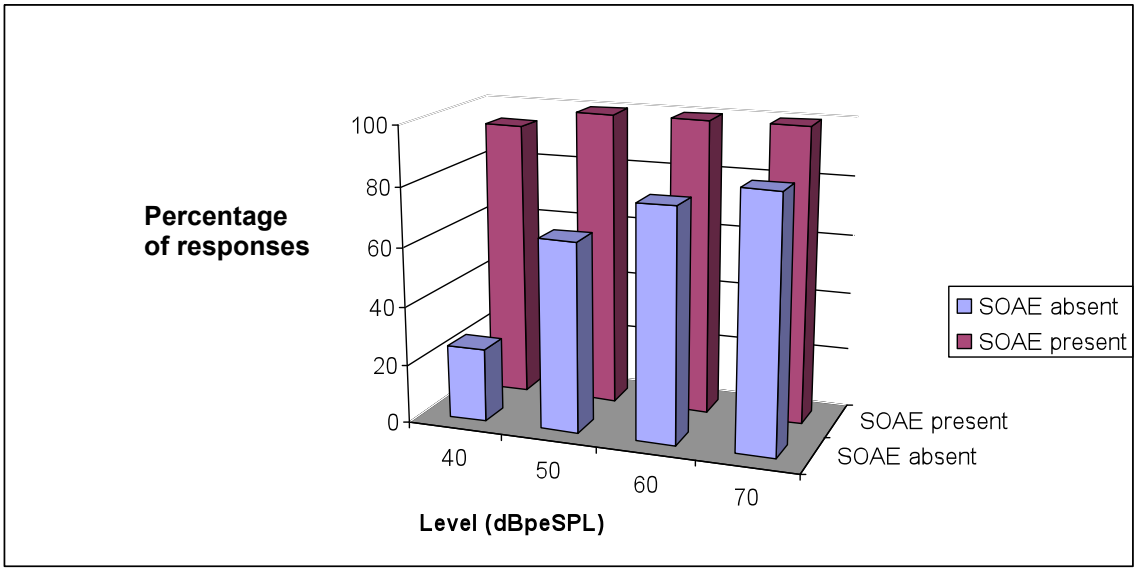


Figure 4.2. *The effect of SOAEs on CEOAEs (obtained at conventional rate 40 clicks/s), for 6-17ms time window at levels tested, correlation>0.5.*

In the majority of ears that responded at all levels SOAEs were present. Conventional responses obtained at a level of 70 dBpeSPL were selected for later comparisons with VSOAEs, and statistical analysis, as this level yielded the most responses when SOAEs were both present and absent.

The volterra slices were extracted from the deconvolved MLS, producing stable and repeatable slices. All responses were subjectively examined for artefacts or contamination. Only those passing this stage were used in the analysis, to ensure the 'quality' of the responses. A 'good' response was then defined as one that had a repeat waveform correlation of >0.5, which is equivalent to an SNR ratio of 1, or SNR= 0 dB. Thus, waveforms were selected if they had a correlation greater than 0.5 in the 2-6 ms time window for the Volterra slices. When analyzing the VSOAE data, the 2-6 ms time window was used in the data analysis, as described above, as in the 6-10 ms time window there were fewer responses. The number of responses

obtained and also the root mean square (RMS) values were computed for this time window and the RMS used as a measure of the response amplitude for the VSOAE second order (S_2) and VSOAE third order (S_3), at the different rates used. The effect of stimulus rate, order and slice number in those subjects both with and without SOAEs was recorded. The distribution of the VSOAE second and third order responses was normal for the second and third order slices. **Figures 4.3, 4.4, 4.5** and **4.6** show the number of VSOAE responses for the second and third order slices of the VSOAEs in those subjects in which SOAEs were absent and those subjects in which they were present.

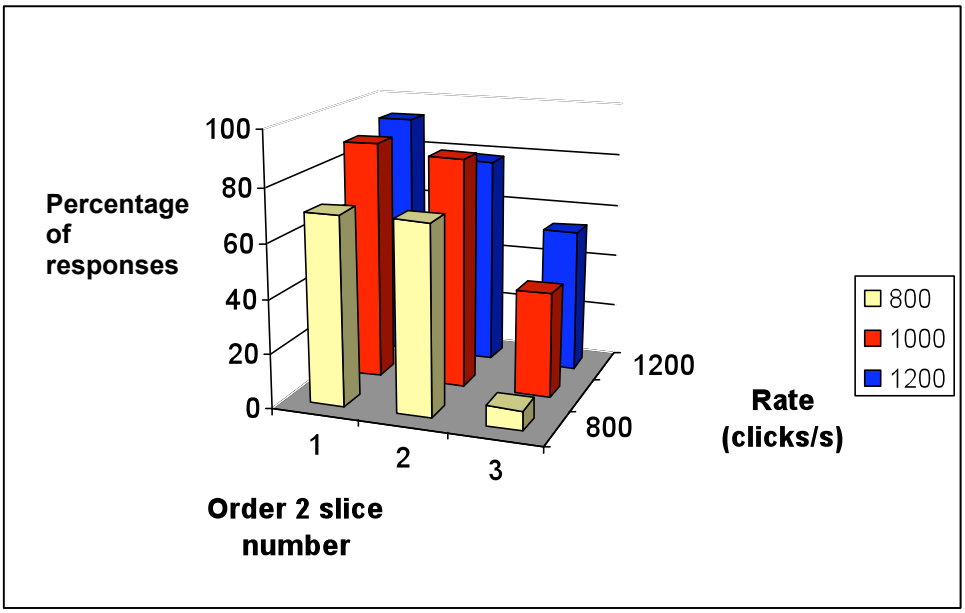


Figure 4.3. *The 2nd order VSOAE variation with stimulus rate, order and slice in responses with correlation>0.5, with no SOAE recorded, time window 2-6ms (percentage of responses= those responses with correlation>0.5/ all responses *100).*

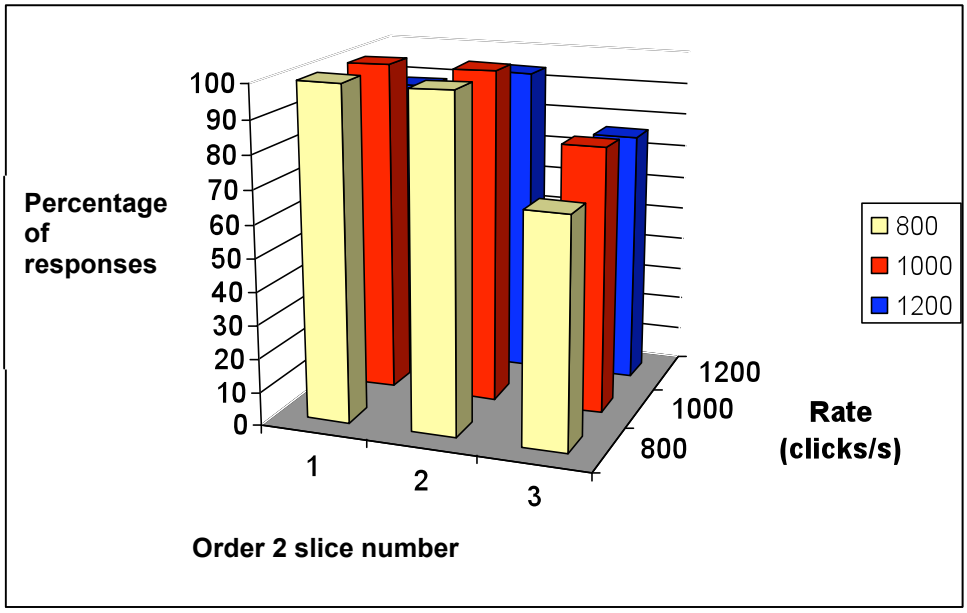


Figure 4.4. *The 2nd order VSOAE variation with stimulus rate, order and slice in responses with correlation>0.5, with SOAE recorded, time window 2-6ms (percentage of responses= those responses with correlation>0.5/ all responses *100).*

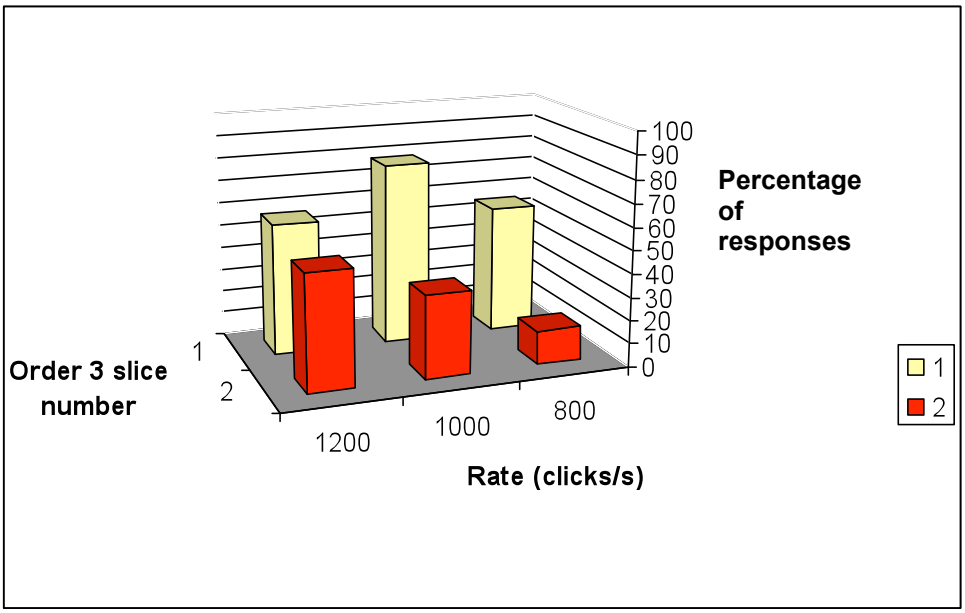


Figure 4.5. *The 3rd order VSOAE variation with stimulus rate, order and slice in responses with correlation>0.5, with no SOAE recorded, time window 2-6ms (percentage of responses= those responses with correlation>0.5/ all responses *100).*

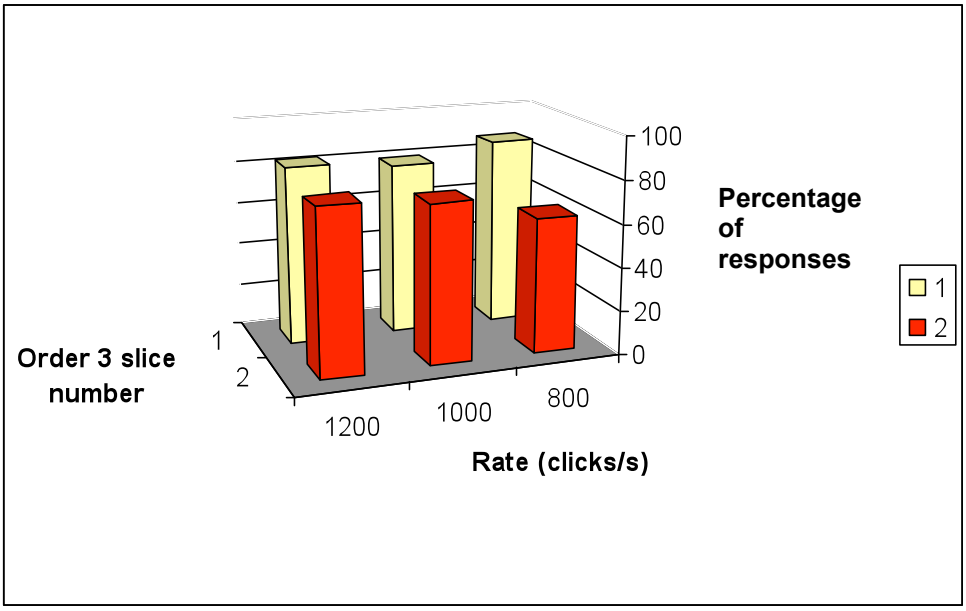


Figure 4.6. *The 3rd order VSOAE variation with stimulus rate, order and slice in responses with correlation>0.5 with SOAE recorded, time window 2-6ms (percentage of responses= those responses with correlation>0.5/ all responses *100).*

It can be seen in **Figures 4.3** to **4.6** that, as is found with conventional CEOAEs (obtained at 40 click/s), subjects with SOAEs show more valid VSOAE responses, especially at higher order/slices. A sufficient number of valid responses were obtained for the VSOAE S_{21}, S_{22} and S_{31}, at a stimulus rate of 1000 clicks/s to analyse the effect of the presence of SOAEs on the root mean square amplitude of these responses. The VSOAE S_{21}, showed the greatest number of responses therefore in statistical analysis this was used in order to assess any significant interaction between rate and the presence of SOAEs. The most valid responses to evaluate the interaction between order and the presence of SOAEs were obtained with VSOAE S_{21} and $S_{31,}$ at a click stimulus rate of 1000 clicks/s, thus have been used in statistical analysis. In the repeated measures ANOVA calculation to assess the significance of the effect between slice and SOAEs, VSOAE S_{21} and S_{22} have been used as these produced a good number of well-correlated responses. Furthermore, at a stimulus rate of 1000 clicks/s, where SOAEs were recorded, slices S_{21} and S_{22} were present for 15 of the 15 conditions, and S_{31} was present for 12 of the 15 conditions. Where

SOAEs were absent, S_{21} was present for 25 out of 28 conditions, S_{22} was present for 24 of the 28 conditions, and slice S_{31} was present for 22 of the 28 conditions.

In order to investigate the effect between SOAEs and the CEOAEs (obtained at the conventional rate), the effect of the SOAE on the amplitude of the CEOAE was investigated (**Figure 4.7**). The distribution for the root mean square amplitude of the CEOAE for the 6-17ms time window was found to be normal.

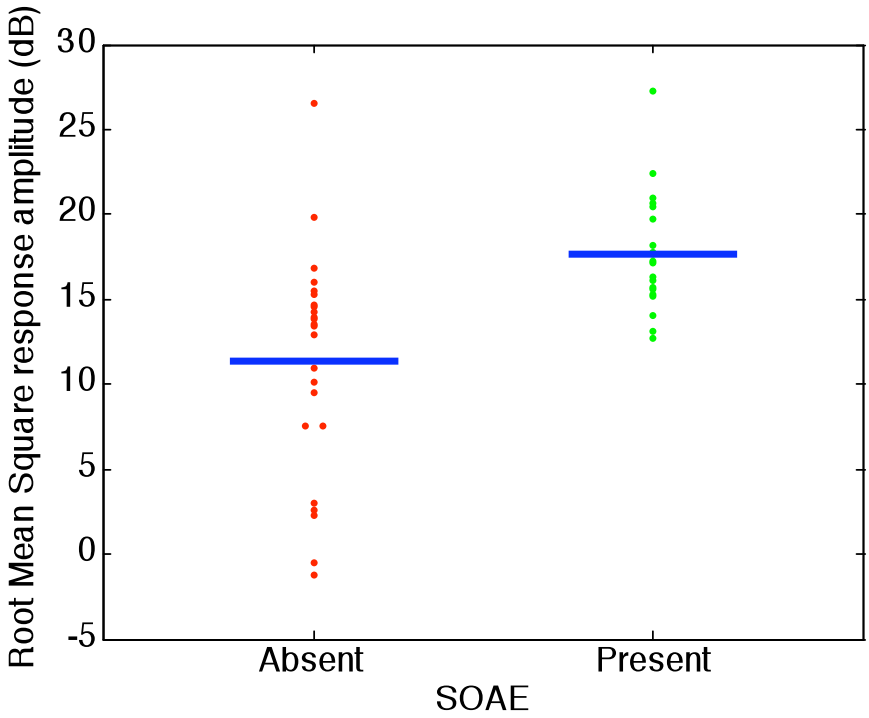


Figure 4.7. *Distribution of the RMS response amplitude for the CEOAE (obtained at 40 clicks/s) for 6-17ms time window, at a stimulus level of 70 dBpeSPL,when SOAEs are absent and present. Valid CEOAE responses analysed with correlation>0.5. Bold lines indicate mean.*

CEOAE	SOAE	N
6-17 ms	Absent	24
	Present	21

Table 4.1. *Number of included responses (N) for CEOAEs (conventional response) at level 70 dBpeSPL, 6-17ms time window.*

Thus overall, those individuals with SOAEs had CEOAEs responses of greater amplitude when compared with those with absent SOAEs. This difference was found to be highly significant on independent samples T test ($p<0.001$).
There was an effect of the level on the interaction of the SOAE with the CEOAE. This can be seen in **Figure 4.8**.

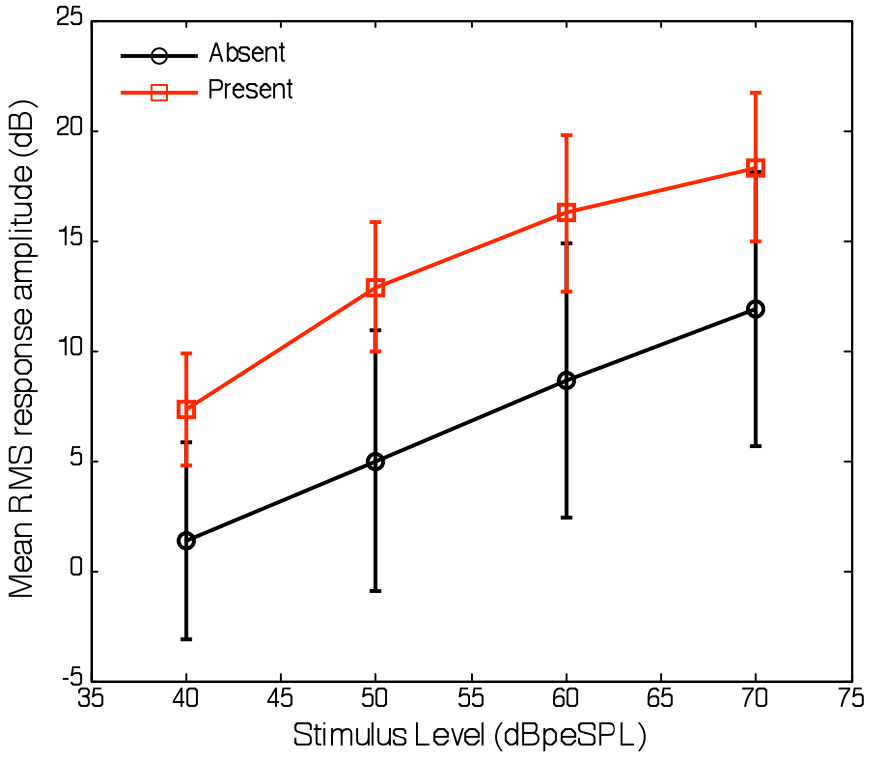
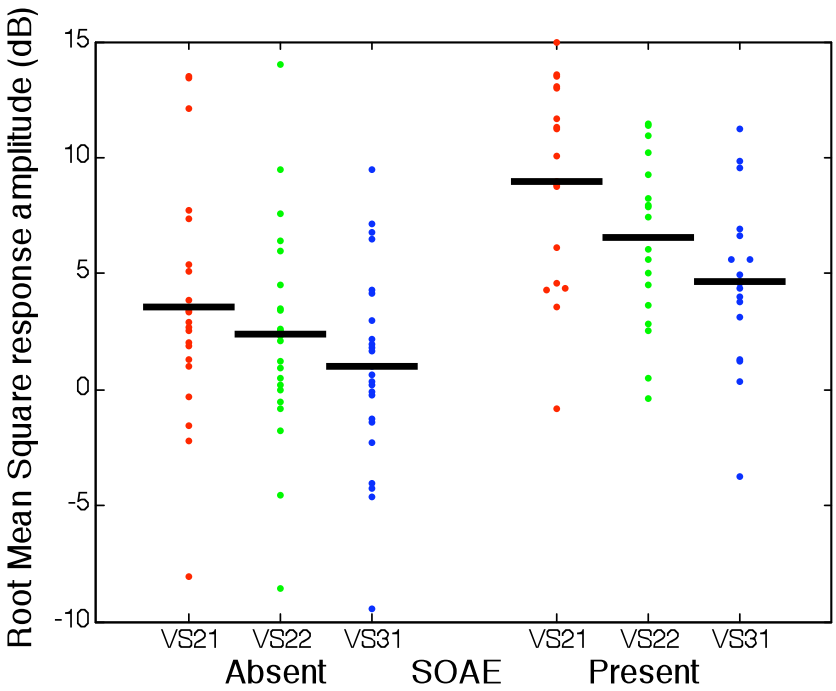


Figure 4.8. *The effect of level on the amplitude of the CEOAEs (conventional rate) in those with and without SOAEs, for the 6-17ms time window. Error bars are shown.*

In both groups the amplitude of the CEOAE increased with an increase in level, as one would expect. The amplitude of the response, as indicated by the mean of the

root mean square amplitude of the response is increased in responses where SOAEs were present. On the application of general linear modelling using univariate analysis of variance, a significant main effect of amplitude of the CEOAE response and SOAE as was shown earlier was found ($p<0.001$); also an effect between the level and the CEOAE response amplitude ($p<0.001$), but there was no interaction between the CEOAE response amplitude, SOAEs and level ($p=0.783$).

There were few outliers for the S_{21}, S_{31} and S_{31} slice waveforms of the VSOAEs (**Figure 4.9** below). The distribution of all of these slices was found to be normal for the 2-6 ms time window at a stimulus rate of 1000 clicks/s. The number of responses analysed for the VSOAEs in both SOAES absent and present groups are shown in **Table 4.2**.



VSOAE	SOAE	N
S_{21}	Absent	24
	Present	19
S_{22}	Absent	24
	Present	19
S_{31}	Absent	24
	Present	19

Table 4.2. *Number of included responses (N) for VSOAEs.*

Figure 4.9. *Distribution of the RMS response amplitude for the 2-6ms time window, at a click stimulus rate of 1000 clicks/s, when SOAEs are absent (SOAE=0) and present (SOAE=1).Valid S_{21}, S_{22} and S_{31} responses analysed with correlation>0.5. Bold lines indicate mean.*

Overall the S_{21} had waveforms of greater amplitude than the S_{22} and S_{31}. The amplitude of the VSOAE was greater in those with SOAEs present for all the chosen slices. The increased amplitude of the VSOAEs, for the S_{21} and S_{22} slices, in the presence of SOAEs was found to be highly significant on an independent samples T-test. In the case of the VSOAE S_{21}, $p<0.0005$, and in the case of VSOAE S_{22}, $p= 0.002$. Taking the Bonferroni correction into account there was no significant difference in the amplitude of the VSOAE S_{31} when SOAES were absent and present ($p= 0.011$).

General linear modelling was applied and on repeated measures ANOVA a significant main effect of rate was found on the VSOAE S_{21} response amplitude in the 2-6ms time window (F= 4.251, p= 0.024). There was no significant interaction between rate and the presence of SOAEs for the VSOAE S_{21} response amplitude for the 2-6ms time window (F= 2.385, p= 0.110). The variation of the mean amplitude with rate is depicted in the **Figure 4.10**.

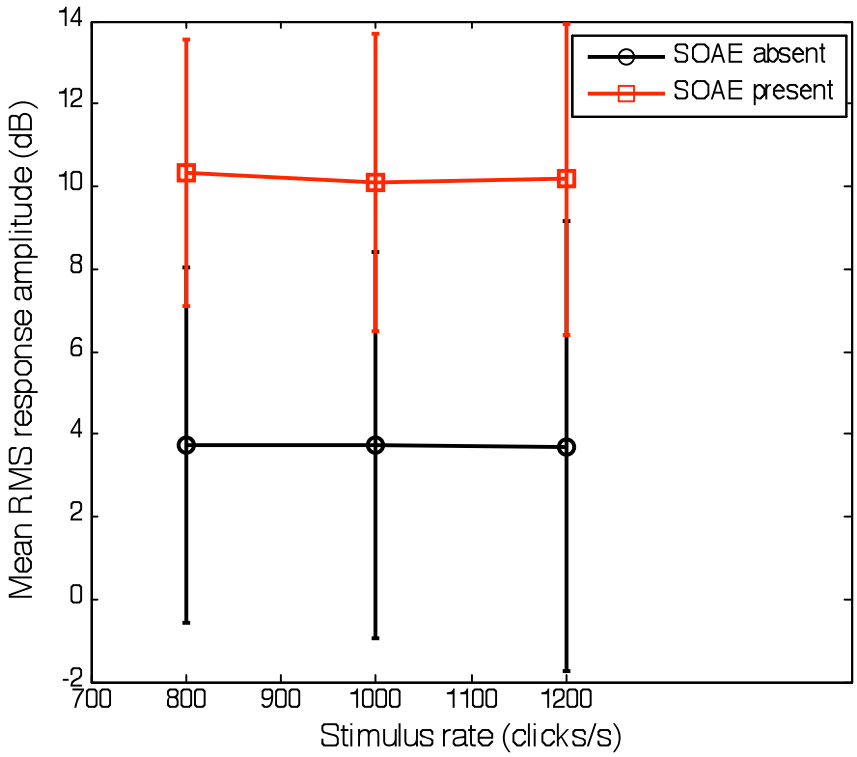


Figure 4.10. *The variation of the mean of the response amplitude with the stimulus rate, for VSOAE 2nd order (slice 1) responses, in those responses where SOAEs were present and absent, for the 2-6 ms time window. Error bars are shown.*

Figure 4.10 shows that the amplitude of the S_{21} decreases with the increased stimulus rates and is greater when SOAEs are present. It also demonstrates that subjects with SOAEs show a greater drop in amplitude with increased rate, but this is not significant.

Repeated measures ANOVA showed a significant effect of order for the VSOAE S_{21} and S_{31} response amplitudes, at a stimulus rate of 1000 clicks/s(F= 77.215, p=

0.000), and also a significant interaction between order and SOAEs for these slices (F=5.307, p= 0.028). This effect is shown below in **Figure 4.11**.

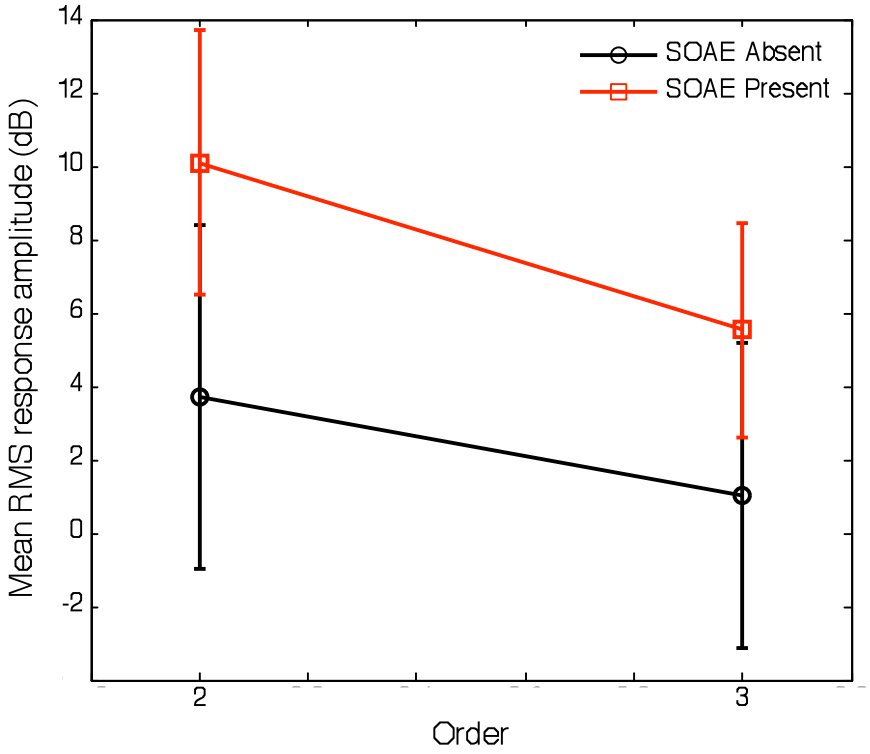


Figure 4.11. *The variation of the mean of the response amplitude with order, for VSOAE 2nd and 3nd orders (slice 1) responses, in those responses where SOAEs were present and absent, at a stimulus rate of 1000clicks/s, for the 2-6 ms time window. Error bars are shown.*

Thus, in the presence of SOAEs there appears to be a greater decline in the VSOAE response amplitude with the higher order responses. In addition once again it can be seen that the VSOAE response amplitude is greater in those subjects where SOAEs are present.

As would be expected, there was a significant effect of slice for the VSOAE S_{21} and S_{22} slice response amplitudes (F= 25.296, p= 0.000). There was no significant interaction between the VSOAE S_{21} and S_{22} slice response amplitudes and SOAEs (F= 1.771, p= 0.192). **Figure 4.12** portrays this result.

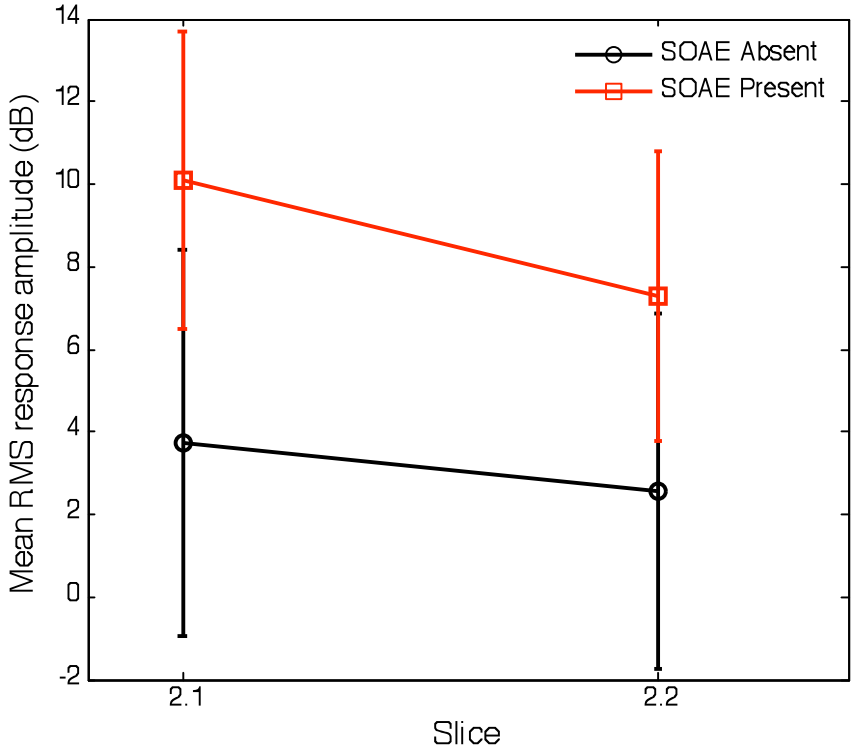


Figure 4.12. *The variation of the mean of the response amplitude with slice, for VSOAE 2nd order slice 1 and 2 responses, in those responses where SOAEs were present and absent, at a stimulus rate of 1000clicks/s, for the 2-6 ms time window. Error bars are shown.*

The VSOAE S_{21} response amplitude is greater than that of the VSOAE S_{22}. As mentioned earlier the response amplitude is greater in the presence of SOAEs.

Thus to summarise, SOAE positive ears have VSOAE responses of greater amplitude than SOAE negative ears and this effect is significant as is the difference in the amplitude between the 2nd and 3rd order (slice 1) responses in SOAE positive and negative ears.

4.4.2) The effect of sex on CEOAEs and VSOAEs

These findings, obviously affect the data analysis. Firstly, SOAE positive ears show VSOAE responses of greater amplitude than SOAE negative ears and therefore females where more SOAEs were present are more likely to have responses of greater amplitude. This has been shown in previous studies.[56] Secondly with fewer males tested in order to ensure data analysis is carried out accurately ears must be balanced for sex, and also in later analysis for side.

The number of valid responses (those with a correlation>0.5) for both male and female subjects was calculated, as were the valid responses in both sexes in those subjects with absent SOAEs. Responses were obtained at levels of 40, 50, 60 and 70 dBpeSPL respectively, as described in the previous section. The 6-17 ms time window was used as this includes the 9-13 ms time window. This is shown in the graphs below (**Figures 4.13 and 4.14**).

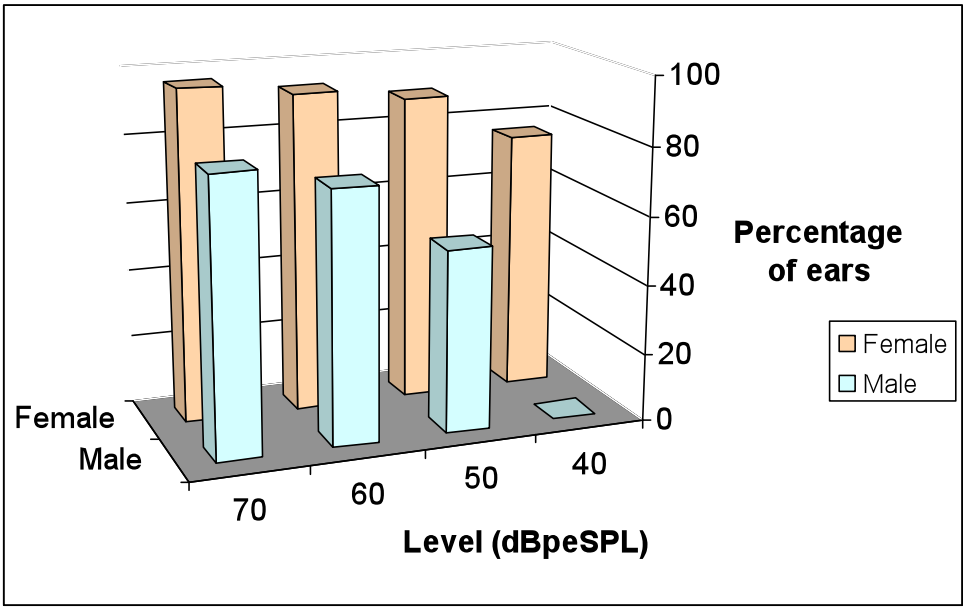


Figure 4.13. *The female and male ears respectively with CEOAE responses at levels tested for the 6-17ms time window (Percentage of female or male ears respectively=those female or male ears respectively responding with correlation>0.5/ all those female or male ears respectively responding *100), for all ears, regardless of SOAE status.*

Therefore, a greater number of valid responses were obtained from female ears, in particular at the lower levels of 40 and 50 dBpeSPL respectively. The highest number of responses was obtained at 70 dBpeSPL; therefore, in later statistical analysis the results obtained at this level have been used. In order to remove any effect the presence of SOAEs may have on the number of valid responses, the number of valid responses for SOAE negative female and male ears was calculated for the same conditions of level and time window.

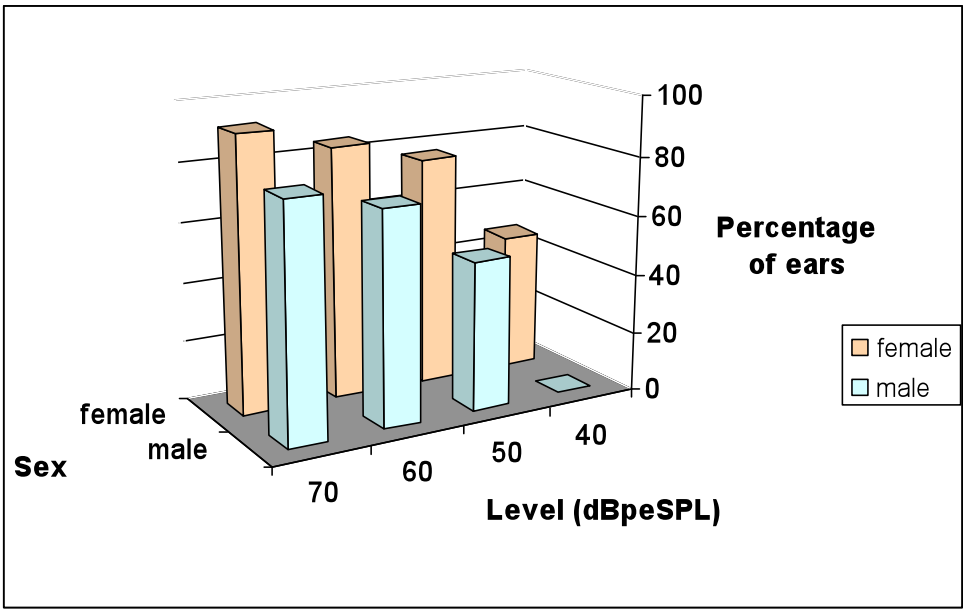


Figure 4.14. *The female and male ears respectively with CEOAE responses at levels tested for the 6-17ms time window with absent SOAEs (Percentage of female or male ears respectively=those female or male ears respectively responding with correlation>0.5/ all those female or male ears respectively responding *100).*

Albeit a female preponderance, there are still more valid responses obtained from females in SOAE negative ears, as the results were calculated as a percentage of all female and male ears responding therefore taking into account the greater number of female subjects.

The percentage of responses with a correlation>0.5 as a function of slice and rate for the 2nd order VSOAE was calculated and is shown in **Figures 4.15** and **4.16** for all female and male ears tested respectively.

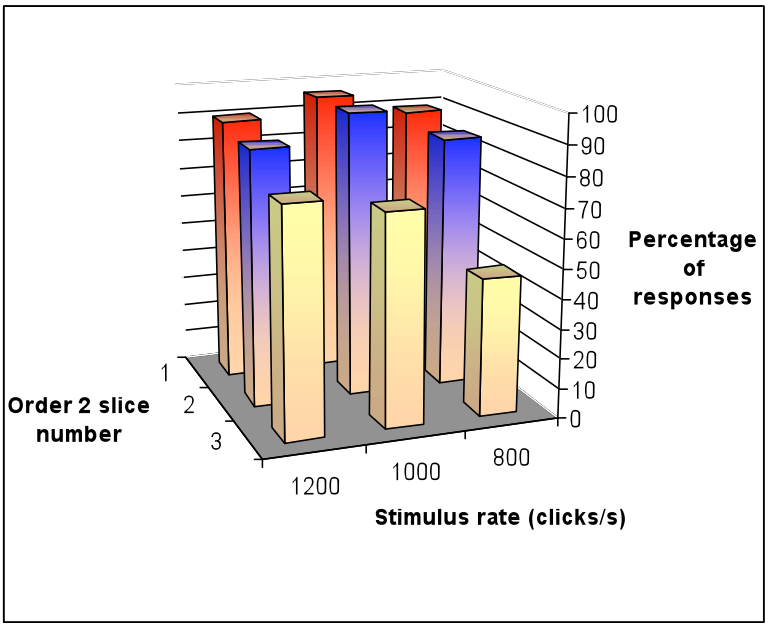


Figure 4.15. *The 2nd order VSOAE variation with stimulus rate and slice in responses with correlation>0.5, for females, time window 2-6ms (percentage of responses= those females with responses with correlation>0.5/ all female responses *100((for that particular measure)).*

It can be seen that a stimulus rate of 1000 clicks/s and S_{21} and S_{22} slice waveforms give the largest number of 'good quality' responses, as such these responses have been used to evaluate the effect between slice and sex. Indeed, at all rates, the S_{21} has the most responses; hence, in statistical calculations this response has been used to assess the effect of amplitude with rate for the sexes.

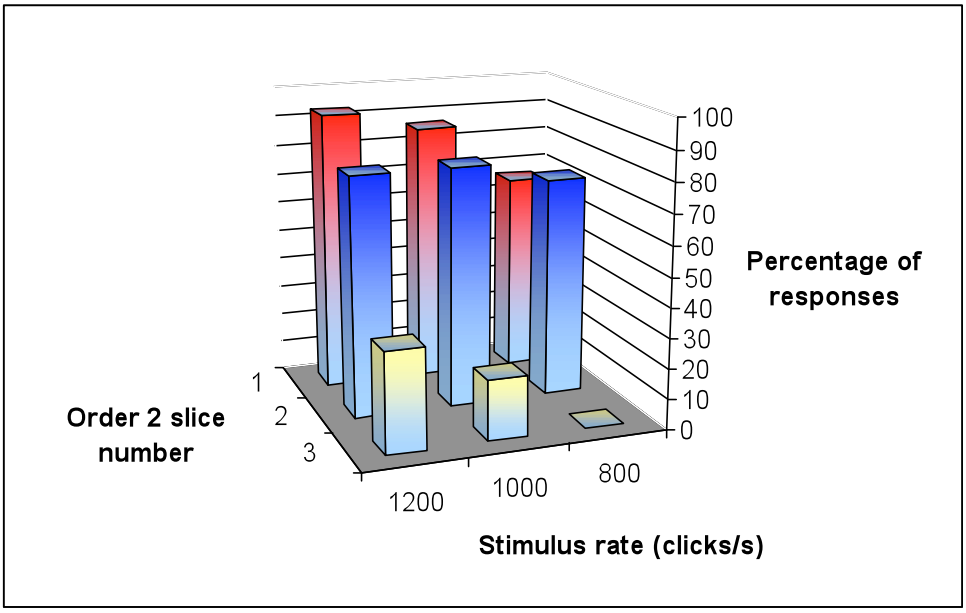


Figure 4.16. *The 2nd order VSOAE variation with stimulus rate and slice in responses with correlation>0.5, for males, time window 2-6ms (percentage of responses= those males with responses with correlation>0.5/ all male responses *100((for that particular measure)).*

In contrast to previous results for overall responses and in females, the largest number of responses for the 2nd order VSOAEs in males was obtained for the S_{21} slice at a stimulus rate of 1200 click/s. However, this result was closely followed by the S_{21} slice at a stimulus rate of 1000 click/s, demonstrating the second largest percentage of 'good quality' responses. Therefore, for valid comparisons the S_{21} slice at a stimulus rate of 1000 click/s has been used in the statistical analysis to assess the interaction of rate and sex. Both these results agree with an earlier study which determined the best stimulus rates to be 1000 or 1500 clicks/s to produce the largest number of 'good' waveforms for the S_2 and S_3 slices.[100] The responses as a function of rate and slice were then calculated in both male and female ears where SOAEs were absent.

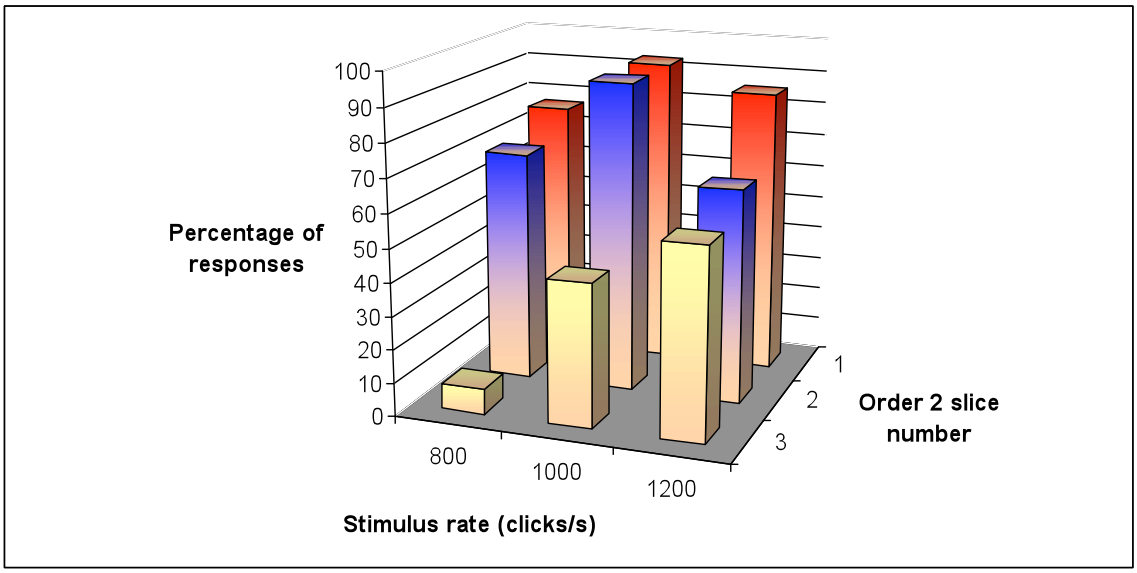


Figure 4.17. *The 2nd order VSOAE variation with stimulus rate and slice in the absence of SOAEs, for responses with correlation>0.5, for females, time window 2-6ms (percentage of responses= those females with responses with correlation>0.5/ all female responses *100((for that particular measure)).*

The number of valid responses present is reduced when subjects with only SOAE negative ears are included.

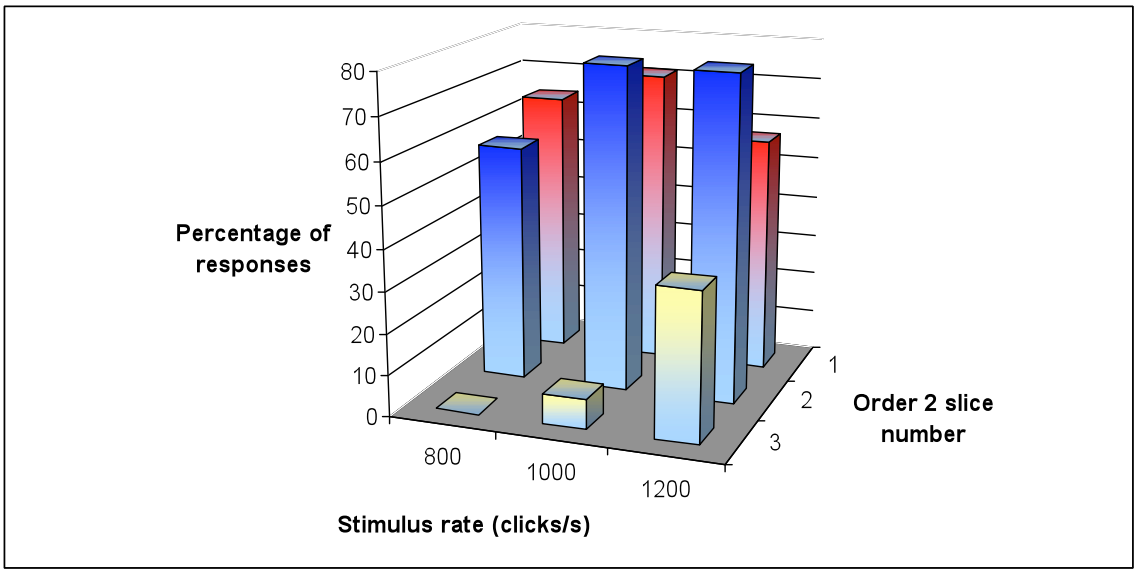


Figure 4.18. *The 2nd order VSOAE variation with stimulus rate and slice in the absence of SOAEs, for responses with correlation>0.5, for males, time window 2-6ms (percentage of responses = those males with responses with correlation>0.5/ all male responses *100 (for that particular measure)).*

The VSOAE 3rd order responses with a correlation>0.5 were recorded as a function of slice and rate for all females and males to show the main effect, and also separately for SOAE negative ears. This is shown in **Figures 4.19** to **4.22** below.

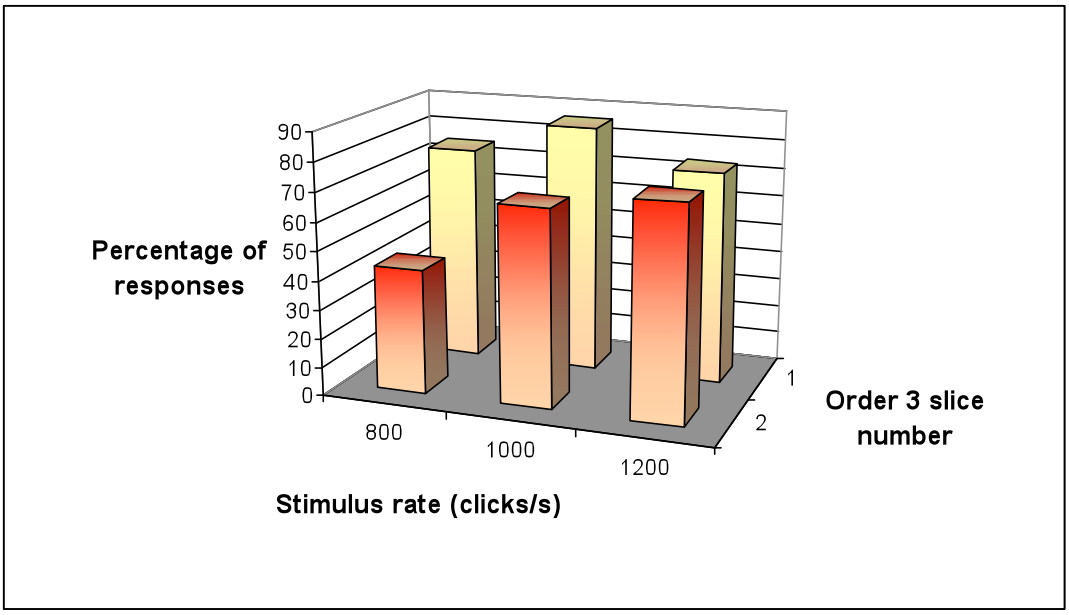


Figure 4.19. *The 3rd order VSOAE variation with stimulus rate and slice in responses with correlation>0.5, for females, time window 2-6ms (percentage of responses= those females with responses with correlation>0.5/ all female responses *100((for that particular measure)).*

There were fewer 'good quality' responses obtained for the 3rd order volterra kernels when compared with the 2nd order volterra kernels for females. It can be seen that for the 3rd order VSOAE S_{31} at a stimulus rate of 1000 clicks/s demonstrates the largest percentage of responses, and thus has been used when assessing the effect between order and sex.

Male responses were then examined in order to once again ensure the best stilmulus rate, order and slice for obtaining 'good quality' responses. **Figure 4.20** shows the results for 3rd order VSOAE.

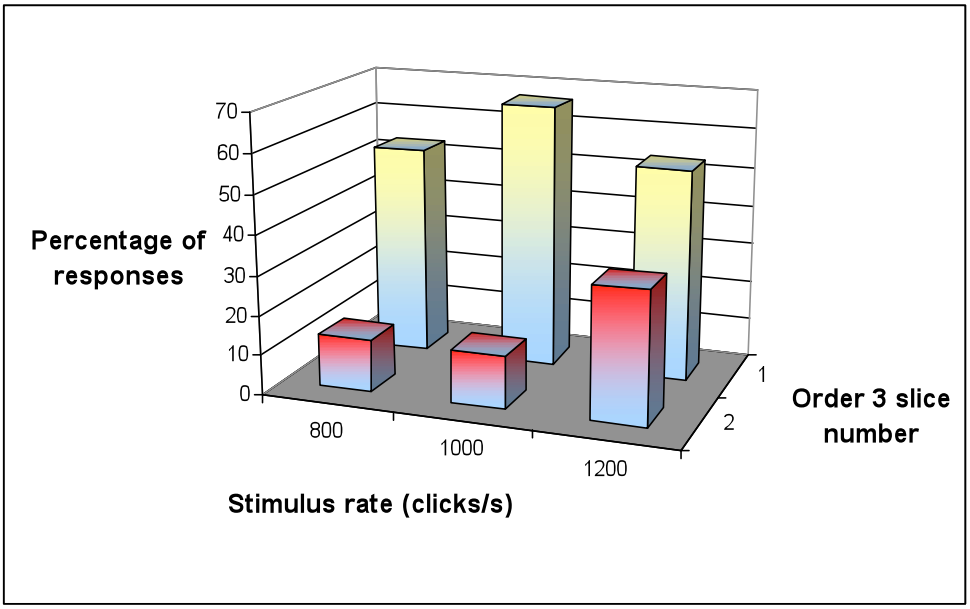


Figure 4.20. *The 3rd order VSOAE variation with stimulus rate and slice in responses with correlation>0.5, for males, time window 2-6ms (percentage of responses= those males with responses with correlation>0.5/ all male responses *100((for that particular measure)).*

The most 'good' responses for males for the 3rd order kernels were obtained for the S_{31} at a stimulus rate of 1000 click/s, in agreement with the results obtained for female responses.

Therefore, the S_{21}, S_{22} and S_{31} at a stimulus rate of 1000 click/s have been chosen for data analysis as described earlier.

The above plots for the 2nd and 3rd order VSOAEs show that females exhibit more valid VSOAE responses than males overall, especially at the higher order and slices. However, when comparing only subjects without SOAEs **(Figures 4.21 & 4.22)** this effect is reduced.

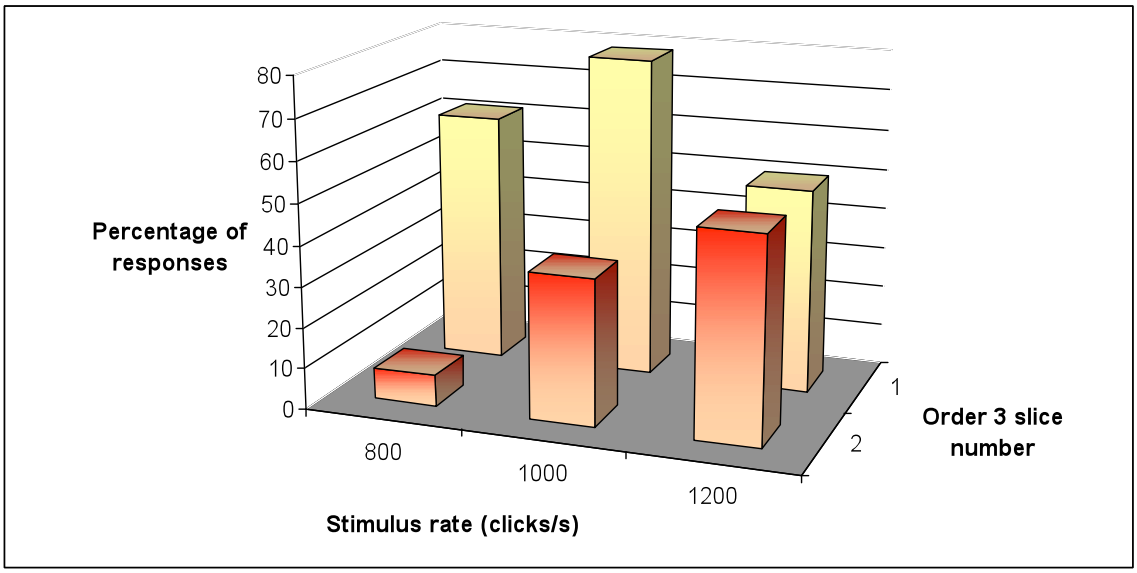


Figure 4.21. *SOAE Absent Females. The 3rd order VSOAE variation with stimulus rate and slice in the absence of SOAEs, for responses with correlation>0.5, for females, time window 2-6ms (percentage of responses= those females with responses with correlation>0.5/ all female responses *100((for that particular measure)).*

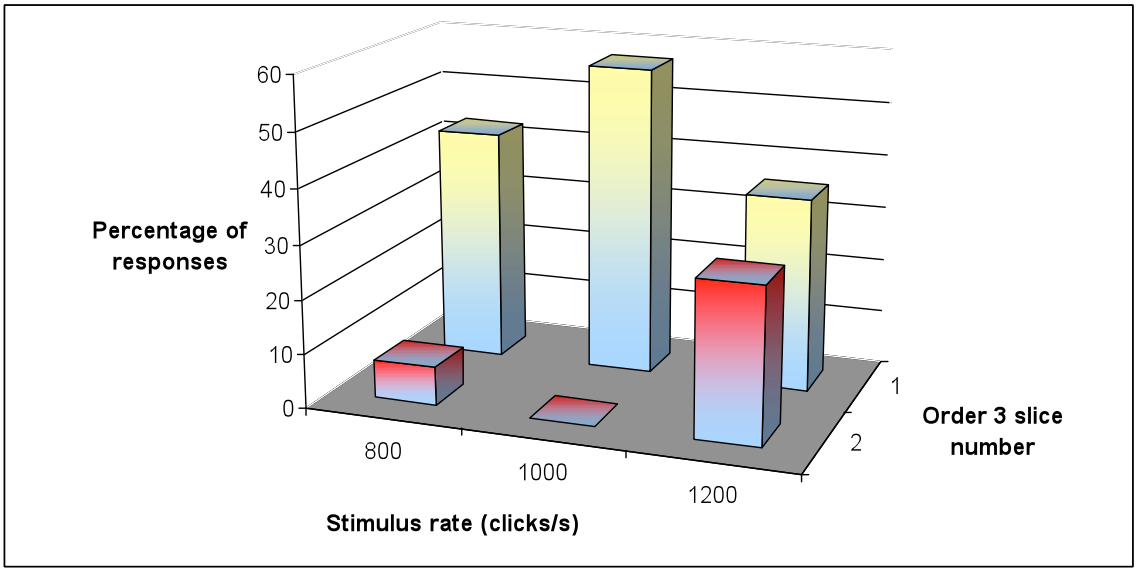


Figure 4.22*. SOAE Absent Males. The 3rd order VSOAE variation with stimulus rate and slice in the absence of SOAEs, for responses with correlation>0.5, for males, time window 2-6ms (percentage of responses= those males with responses with correlation>0.5/ all male responses *100((for that particular measure)).*

The RMS amplitudes of the selected responses (CEOAE at 70 dBpeSPL, 6-17 ms time window; and VSOAEs $S_{21,}$ S_{22} and S_{31} at a stimulus rate of 1000 click/s) for all females and males (regardless of SOAE status) and in those with absent SOAEs were then plotted and are illustrated in **Figures 4.23-4.30** below.

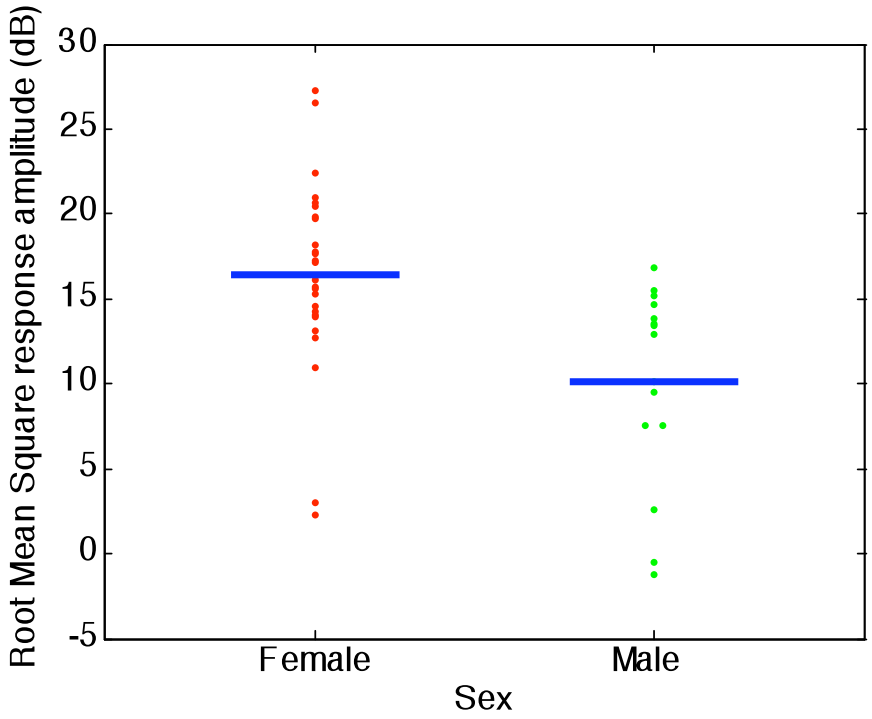


Figure 4.23. *RMS amplitude for CEOAE (40 click/s) all. Distribution of the RMS response amplitude for the CEOAEs 6-17 ms time window, at a click at stimulus level 70 dBpeSPL, for all responses analysed with correlation>0.5. Bold lines indicate mean.*

CEOAE	Sex	N
	Female	29
	Male	15

Table 4.3. *Number of included responses (N) for CEOAE for all females and males.*

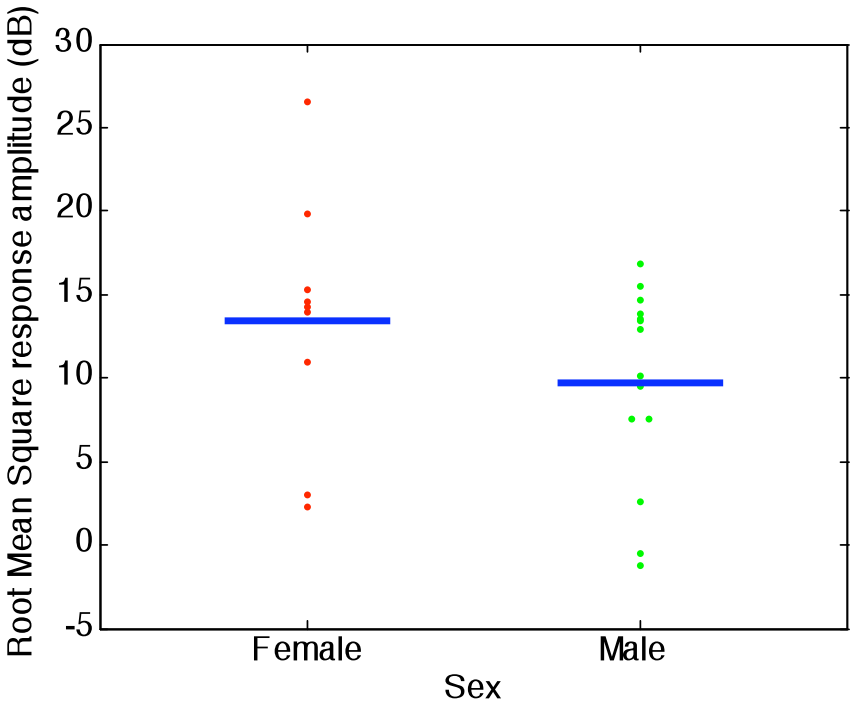


Figure 4.24. *RMS amplitude for CEOAE (40 click/s), SOAE absent. Distribution of the RMS response amplitude for the 6-17ms time window, at a stimulus level of 70 dBpeSPL, when SOAEs are absent, responses analysed with correlation>0.5. Bold lines indicate mean.*

CEOAE	Sex	N
	Female	9
	Male	14

Table 4.4. *Number of included responses (N) for CEOAE, in ears with absent SOAEs.*

As can be seen in **Figures 4.23** and **4.24** the root mean square amplitude of the CEOAE obtained at the conventional rate is greater in females than in males in agreement with our previous results (Chapter 3) and those found in previous studies with CEOAEs. These figures also show that females have responses of greater amplitude than males in subjects in whom SOAEs were not present. The difference in amplitude between the female and male emissions, with females having emissions of greater amplitude than males was found to be approach significance when all subjects were included (p= 0.007), taking into account the Bonferroni correction.

When SOAEs were absent, although there was a difference as demonstrated in **Figure 4.24**, this was not significant (p= 0.328). The results are summarised in **Table 4.5** below.

CEOAE	Sex	N	t	df	Sig.(2-tailed)
All responses	1 2	29 15	2.877	38	0.007
Responses in SOAE negative ears	1 2	9 14	1.007	17	0.328

Table 4.5. *Comparison of Independent samples t-test results for interaction between sex and CEOAE, where equal variance is assumed and sex 1=female and sex 2=male. (Bonferroni correction p= 0.004).*

Following the investigation of the sex differences for the CEOAEs in this second series of experiments, the root mean square amplitude of the VSOAE $S_{21,}$ S_{22} and S_{31} at a stimulus rate of 1000 click/s were examined, and any significant differences in amplitude between the sexes revealed. **Figures 4.25- 4.30** illustrate the differences in the root mean square amplitude between females and males for all ears and in only those ears with absent SOAEs. The number of responses included has been recorded in **Tables 4.6- 4.11.**

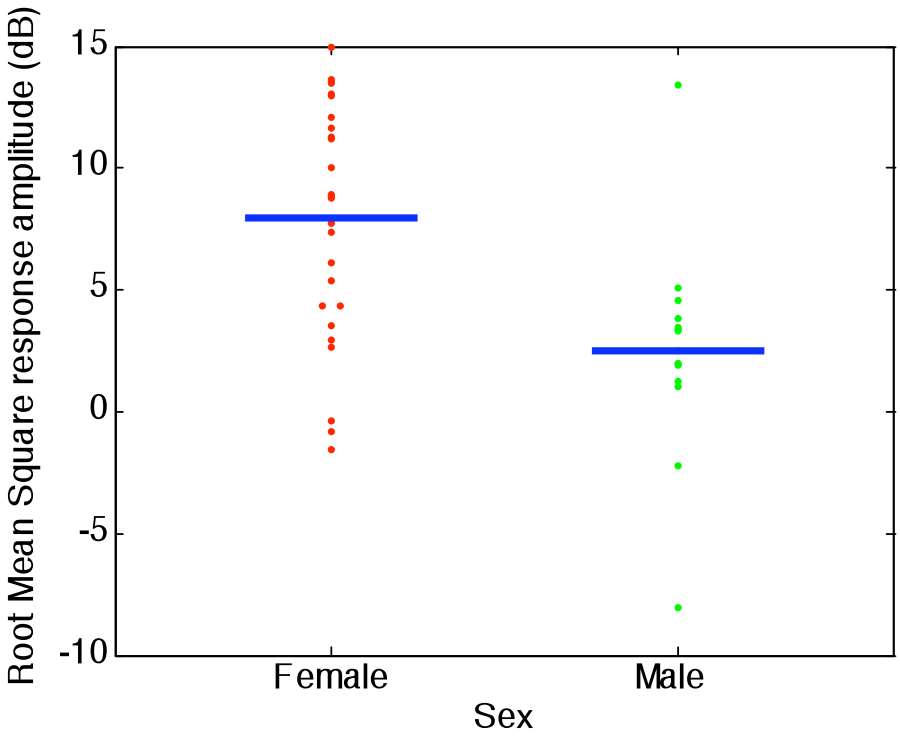


Figure 4.25. *Distribution of the RMS response amplitude for the VSOAE $S_{21,}$ for all valid responses (corr>0.5), at a click stimulus rate of 1000 clicks/s, 2-6ms time window. Bold lines indicate mean.*

VSOAE S_{21}	**Sex**	**N**
	Female	27
	Male	15

Table 4.6. *Number of included responses (N) for VSOAE $S_{21,}$ for all females and males.*

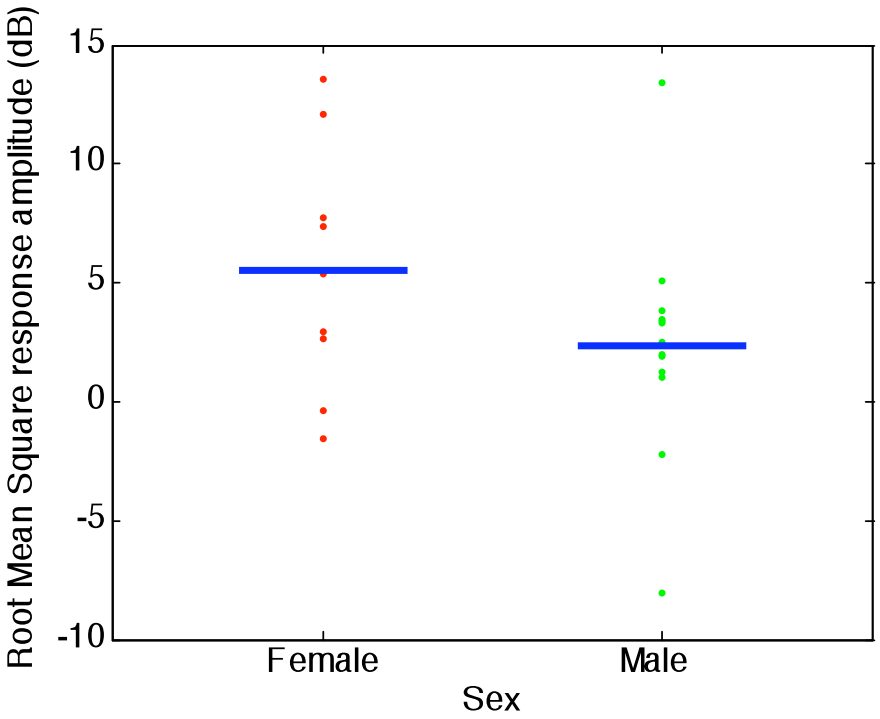


Figure 4.26. *Distribution of the RMS response amplitude for the VSOAE $S_{21,}$ when SOAEs are absent, for valid responses (corr>0.5), at a click stimulus rate of 1000 clicks/s, 2-6ms time window. Bold lines indicate mean.*

VSOAE S_{21}	**Sex**	**N**
	Female	9
	Male	14

Table 4.7. *Number of included responses (N) for VSOAE $S_{21,}$ in ears with absent SOAEs.*

In both cases in all ears and those with absent SOAEs for the VSOAE S_{21} the amplitude of the female response is greater than that of the male response. This in spite of only 9 female responses and 14 male responses in SOAE negative ears. Furthermore, it can also be seen that the amplitude of the response is decreased in ears with absent SOAEs.

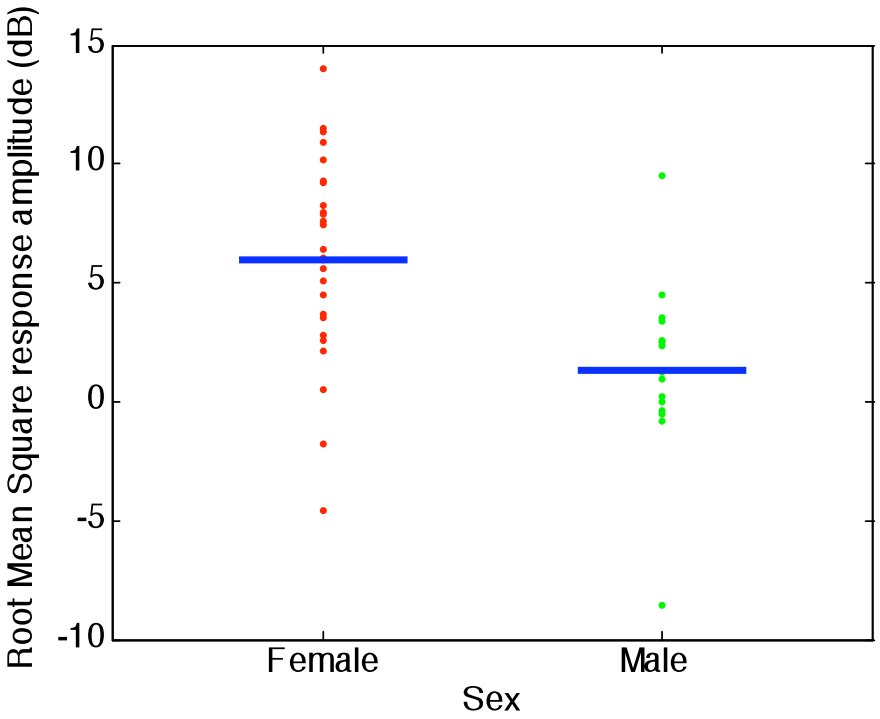


Figure 4.27. *Distribution of the RMS response amplitude for the VSOAE S_{22}, for all valid responses (corr>0.5), at a click stimulus rate of 1000 clicks/s, 2-6ms time window. Bold lines indicate mean.*

VSOAE S_{22}	**Sex**	**N**
	Female	27
	Male	15

Table 4.8. *Number of included responses (N) for VSOAE S_{22}, for all females and males.*

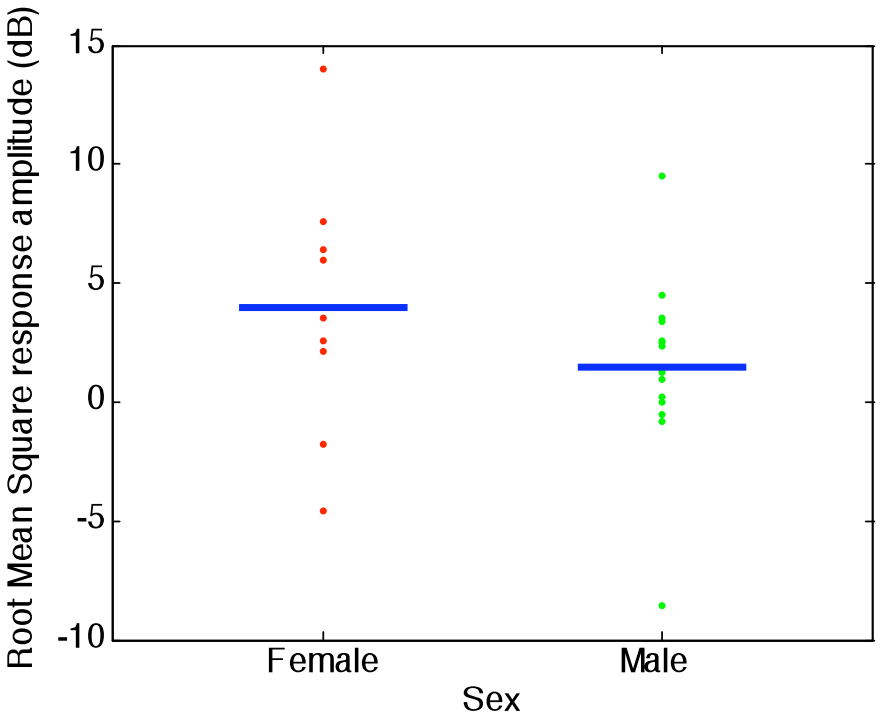


Figure 4.28. *Distribution of the RMS response amplitude for the VSOAE S_{22}, when SOAEs are absent, for valid responses (corr>0.5), at a click stimulus rate of 1000 clicks/s, 2-6ms time window. Bold lines indicate mean.*

VSOAE S_{22}	**Sex**	**N**
	Female	9
	Male	14

Table 4.9. *Number of included responses (N) for VSOAE S_{22}, in ears with absent SOAEs.*

These plots **(Figures 4.27 & 4.28)** also show that females have emissions of greater amplitude than males for the VSOAE S_{22}, for all valid responses and in SOAE negative ears. The female and male ear amplitudes obtained for the VSOAE S_{22} is smaller in SOAE-negative ears and also is less than that obtained for the VSOAE S_{21}.

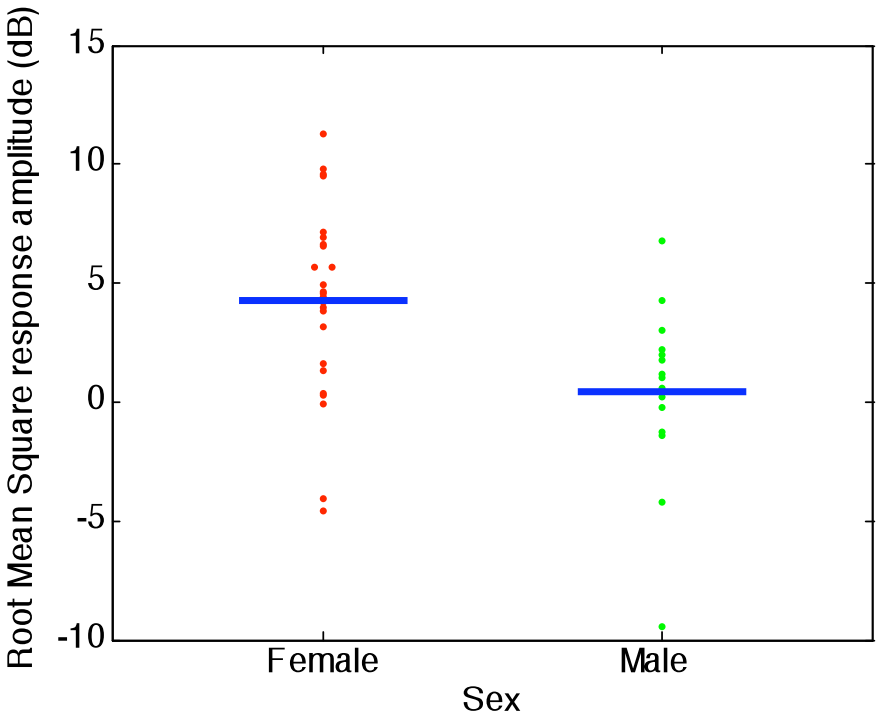


Figure 4.29. *Distribution of the RMS response amplitude for the VSOAE $S_{31,}$ for all valid responses (corr>0.5), at a click stimulus rate of 1000 clicks/s, 2-6ms time window. Bold lines indicate mean.*

VSOAE S_{31}	**Sex**	**N**
	Female	26
	Male	15

Table 4.10. *Number of included responses (N) for VSOAE S_{31}, for all females and males.*

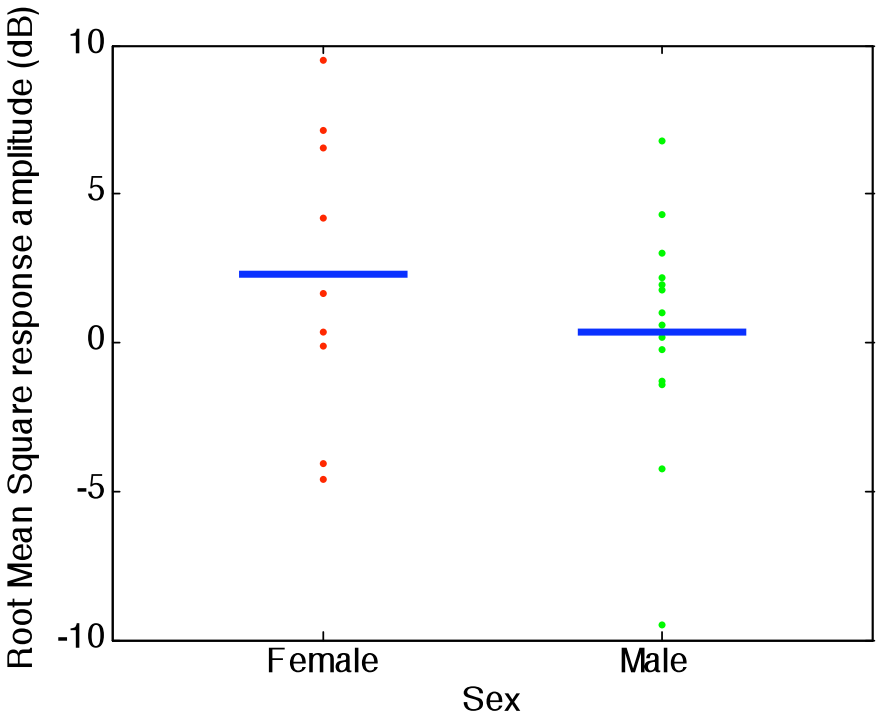


Figure 4.30. *Distribution of the RMS response amplitude for the VSOAE $S_{31,}$ when SOAEs are absent, for all valid responses (corr>0.5), at a click stimulus rate of 1000 clicks/s, 2-6ms time window. Bold lines indicate mean.*

VSOAE S_{31}	**Sex**	**N**
	Female	9
	Male	14

Table 4.11. *Number of included responses (N) for VSOAE S_{31}, in ears with absent SOAEs.*

In agreement with the results obtained for the VSOAE S_{21} and S_{22}, for the VSOAE S_{31} the amplitude of the female response is larger than that of males where all responses are analysed, but this does not appear to be the case when only ears with absent SOAEs are included. When SOAEs are absent there appears to be no difference in the median value between males and females (**Figure 4.30**).

The significance of any differences in amplitude between females and males for all the analysed VSOAE responses and in SOAE-negative ears, that were well

correlated, was undertaken using Independent samples t-test. These results are shown in **Table 4.12** below.

VSOAE	Condition	Sex	N	t	df	Sig.(2-tailed)
S_{21}	All responses	1 2	27 15	3.699	32.786	**0.001**
S_{21}	*Responses in SOAE negative ears*	1 2	9 14	1.054	19	0.305
S_{22}	All responses	1 2	27 15	3.228	36	**0.003**
S_{22}	*Responses in SOAE negative ears*	1 2	9 14	1.023	18	0.320
S_{31}	All responses	1 2	26 15	1.800	32	0.081
S_{31}	*Responses in SOAE negative ears*	1 2	9 14	0.378	11.795	0.712

Table 4.12. *Comparison of Independent samples t-test results for interaction between sex and VSOAEs. Bold print in last column indicates significant result.*

The table shows that for the VSOAE S_{21} and S_{22} for all female and male responses there is a significant difference between females and males, with females having responses of larger amplitude than males. When SOAEs are not present, the female/male diversity is not significant for all three VSOAE slices.

To elucidate the interaction between VSOAEs and sex further repeated measures ANOVA was embarked on. Initially the effect of rate and sex was explored looking at the VSOAE S_{21} at all stimulus rates (800 clicks/s, 1000 clicks/s and 1200 clicks/s). There was a significant effect of rate ($F= 6.860$, $p= 0.002$) and significant interaction between rate and sex ($F= 3.342$, $p= 0.042$). The main effect of rate and the interaction between rate and sex is demonstrated in **Figure 4.31**.

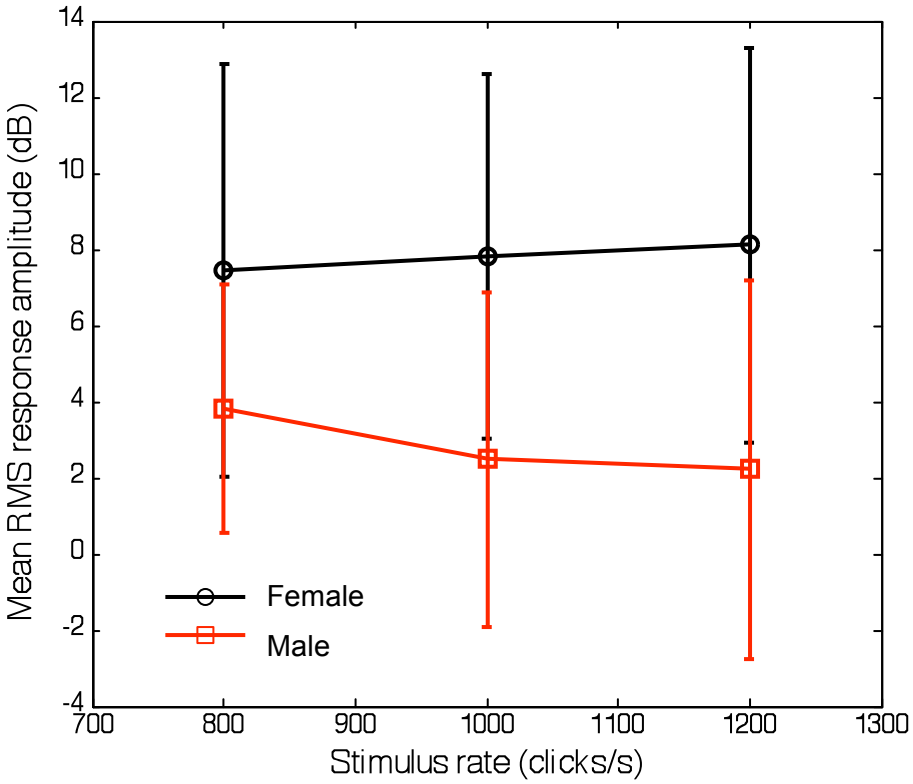


Figure 4.31. *The variation of the mean of the response amplitude with the stimulus rate, for VSOAE 2nd order (slice 1) responses for females and males respectively, in those responses where SOAEs were present and absent, for the 2-6 ms time window. Error bars are shown.*

It can be deduced from **Figure 4.31** that in addition to females having greater amplitude responses, that as the stimulus rate increases, there is a decline in the amplitude of the response in males as occurs with CEOAEs. There is a greater decline in amplitude with increasing stimulus rate in males between stimulus rates of 800 and 1200 clicks/s, as can be seen from the steeper slope.

VSOAE S_{21} and S_{31}, at a stimulus rate of 1000 clicks/s were selected to assess the interaction between rate and order. On general linear modeling, repeated measures ANOVA revealed a significant main effect of order ($F= 46.972$, $p<0.0005$), and interaction of order and sex ($F= 4.083$, $p= 0.052$). This effect is shown in **Figure 4.32**.

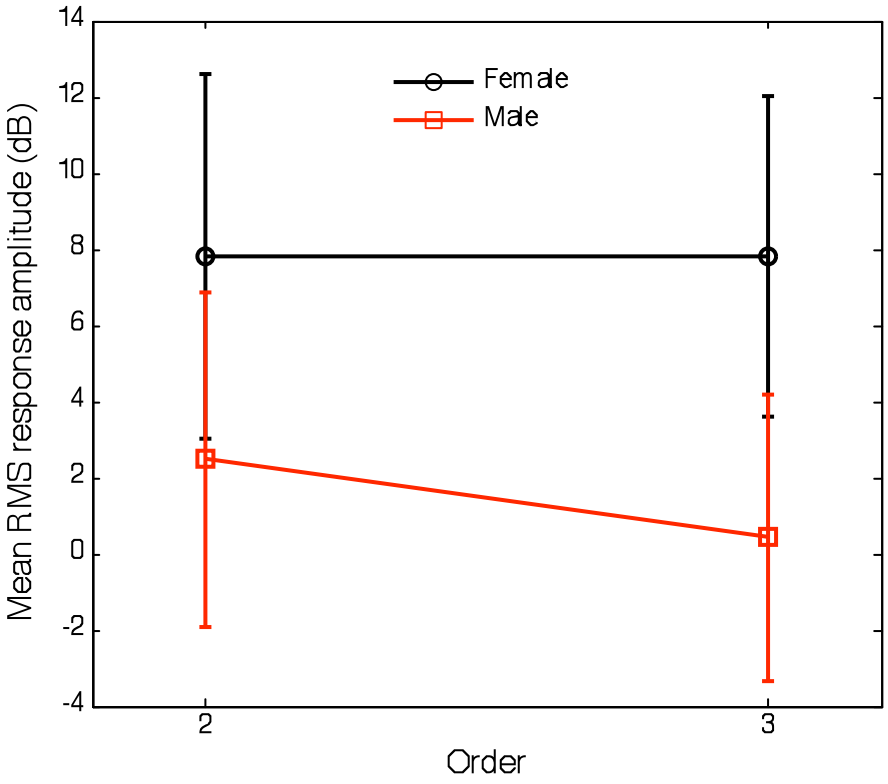


Figure 4.32. *The variation of the mean of the response amplitude with the order, for VSOAE 2nd and 3rd orders (slice 1) responses for all females and males respectively, in those responses where SOAEs were present and absent, at a stimulus rate of 1000clicks/s, for the 2-6 ms time window. Error bars are shown.*

It can be seen from **Figure 4.32** that there is a greater decline in the amplitude of the response with the higher order in males compared with females. Females' emissions are larger than male emissions for both orders.

The interaction between slice and sex using the chosen slices (VSOAE S_{21} and S_{22}, at a stimulus rate at 1000 clicks/s) showed a significant main effect of slice ($F= 16.550$, $p<0.0005$), but no interaction between slice and sex ($F= 0.281$, $p= 0.600$).

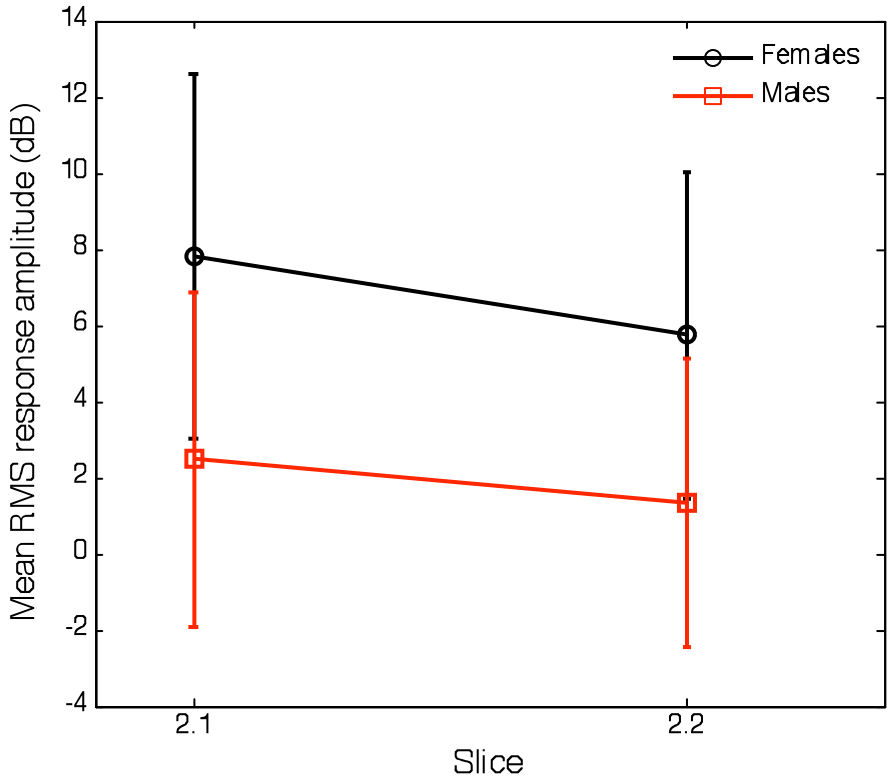


Figure 4.33. *The variation of the mean of the response amplitude with slice, for VSOAE 2nd order slice 1 and 2 responses for females and males respectively, in those responses where SOAEs were present and absent, at a stimulus rate of 1000clicks/s, for the 2-6 ms time window. Error bars are shown.*

The VSOAE 2nd order slice 1 has a greater amplitude than the slice 2 as shown in **Figure 4.33**; this is the main effect of slice.

4.4.3) The effect of side on CEOAEs and VSOAEs

We previously demonstrated a female right/left asymmetry in the auditory periphery (Chapter 3). In this next series of experiments we re-examined the CEOAE ear asymmetry obtained at the conventional rate, using the MLS machinery. Moreover, we examined the VSOAEs to establish if the ear side differences found with conventional CEOAEs applied to these non-linear temporal interaction components.

At the outset, in all cases, all valid responses (correlation>0.5) were analysed, and subsequently those where SOAEs were absent were analysed. SOAEs were present in 30 out of 45 ears; therefore, by excluding SOAEs far fewer responses are analysed which impacts on the results.

Initially the number of valid responses for the CEOAE, for all right and left ears was calculated and is shown in **Figure 4.34**. Following on from this the number of valid responses for the CEOAE in all right and left ears with absent SOAEs was calculated and is shown in **Figure 4.35**. The time window selected was the 6-17 ms time window as this contained the 9-13 ms time window and captured more of the response.

Figure 4.34 illustrates that there were a similar number of 'good quality' responses obtained from both right and left ears. It can also be seen the greatest number of responses were obtained at 70 dBpeSPL for left ears, and at both 60 and 70 dBpeSPL for right ears.

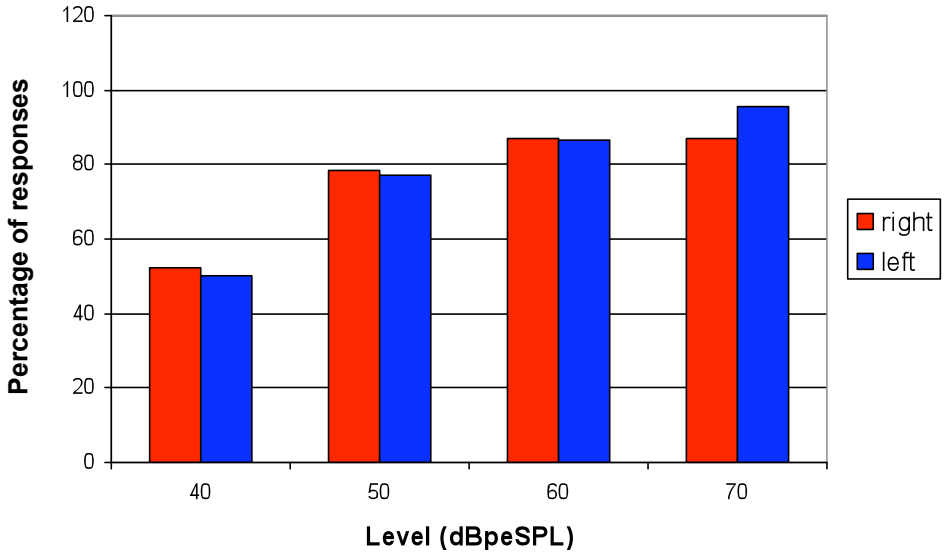


Figure 4.34. *The right and left ears respectively with CEOAE responses (obtained at the conventional rate of 40 clicks/s), at all levels tested for the 6-17ms time window (Percentage of responses ((right or left respectively))=those right or left ears respectively responding with correlation>0.5/ all those right or left ears respectively responding *100).*

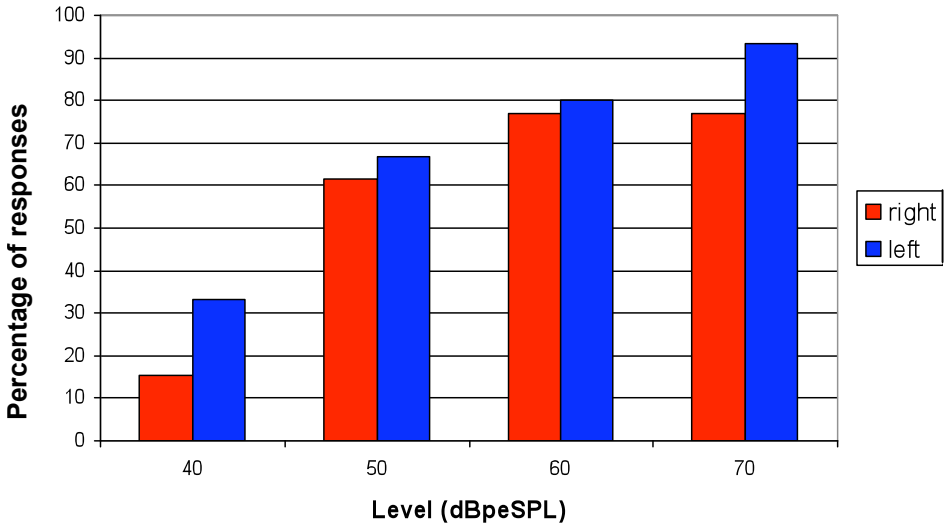


Figure 4.35. *The right and left ears respectively with CEOAE responses (obtained at the conventional rate of 40 clicks/s) at all levels tested for the 6-17ms time window, with absent SOAEs (Percentage of responses ((right or left respectively))=those right or left ears respectively responding with correlation>0.5/ all those right or left ears respectively responding *100).*

In SOAE-negative ears there appear to be more responses obtained from left ears than right ears as can be deduced from **Figure 4.35**. The most responses were obtained at 70 dBpeSPL; therefore, this level has been selected for statistical analysis for the CEOAE ear side difference in both SOAE negative ears and for all ears.

The same process of recording the percentage of well correlated responses (correlation>0.5) in all right and left ears, and for ears where SOAEs were absent was repeated for the VSOAE 2nd order (slices 1, 2, 3) and 3rd order (slices 1 & 2).
The 2-6 ms time window was selected as this contains the most prominent part of the response. These results are represented in **Figures 4.36-4.43**.

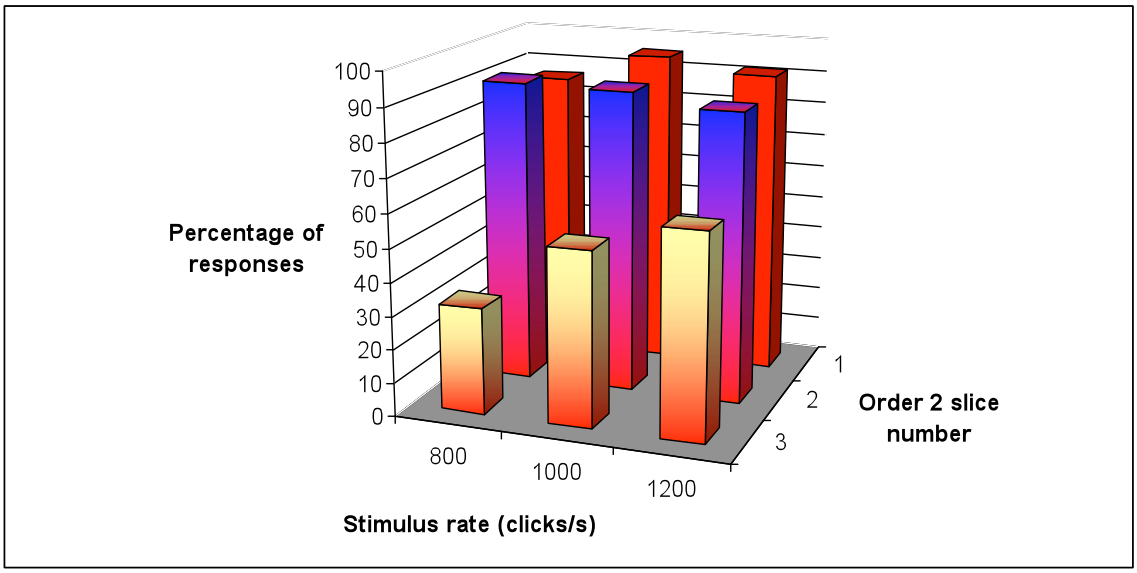


Figure 4.36. *All right ears respectively with VSOAE 2nd order responses at stimulus rates tested for the 2-6ms time window (Percentage of responses= those right ear responses with correlation>0.5/ all right ear responses *100((for that particular measure)).*

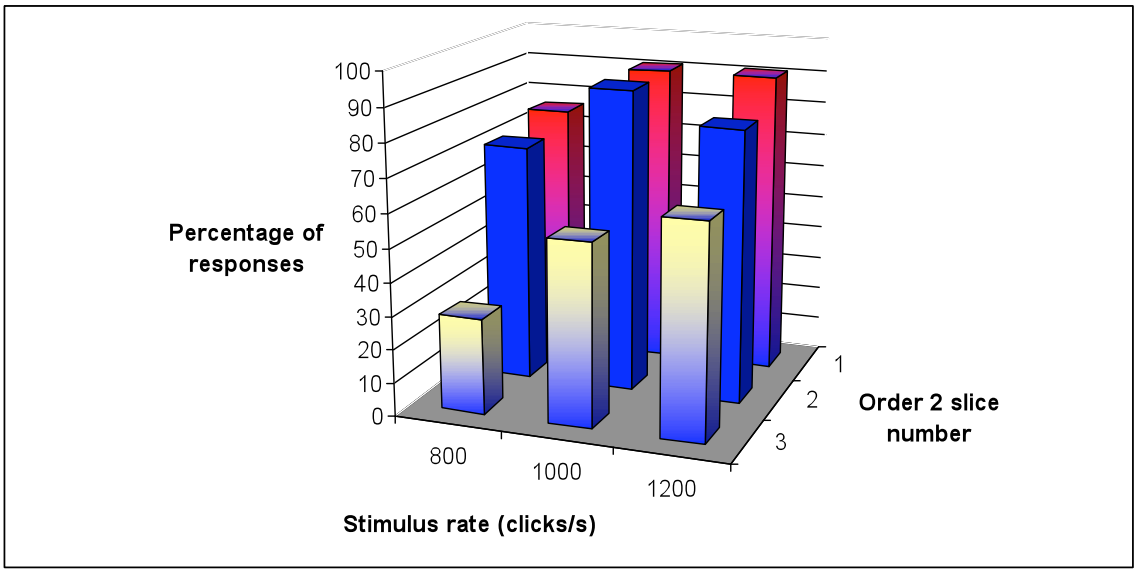


Figure 4.37. *All left ears with VSOAE 2nd order responses at stimulus rates tested for the 2-6ms time window (Percentage of responses= those left ear responses with correlation>0.5/ all left ear responses *100((for that particular measure)).*

For right and left ears (**Figures 4.36 & 4.37**) the most responses were obtained for the VSOAE S_{21} in most instances, the exception being in the right ear at a stimulus rate of 800 clicks/s. The majority of responses were obtained at a stimulus rate of 1000 clicks/s. For the second order the VSOAE S_{21} and S_{22} produced more

acceptable responses than the VSOAE S_{23}. The results obtained in SOAE negative ears showed these same effects, but with fewer responses overall. This can be seen in **Figures 4.38 and 4.39**.

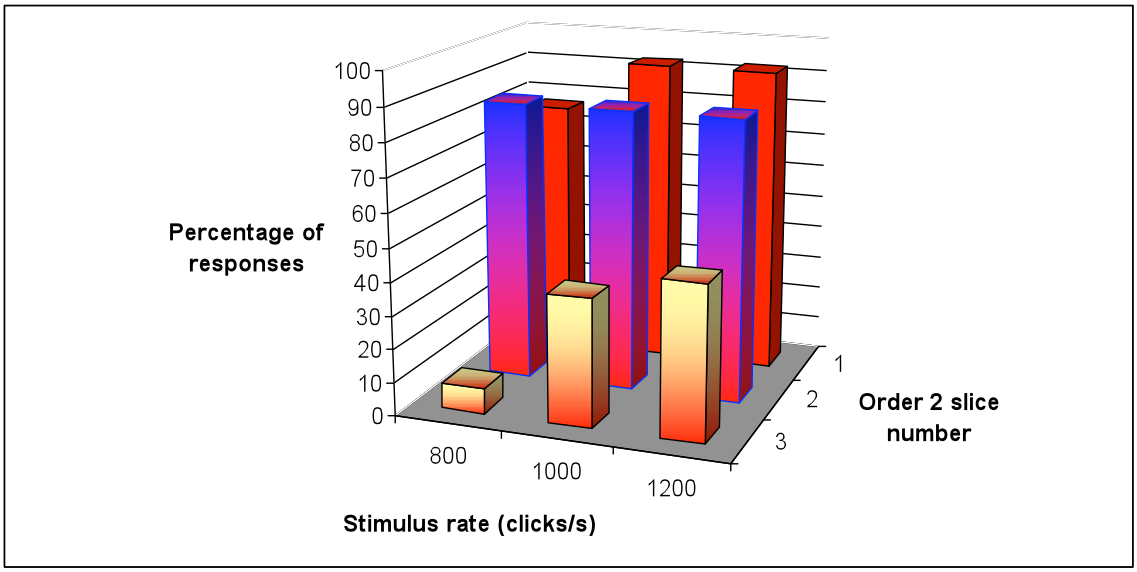


Figure 4.38. *Right ears with absent SOAEs. VSOAE 2nd order responses at stimulus rates tested for the 2-6ms time window (Percentage of responses= those right ear responses with correlation>0.5/ all right ear responses *100((for that particular measure)).*

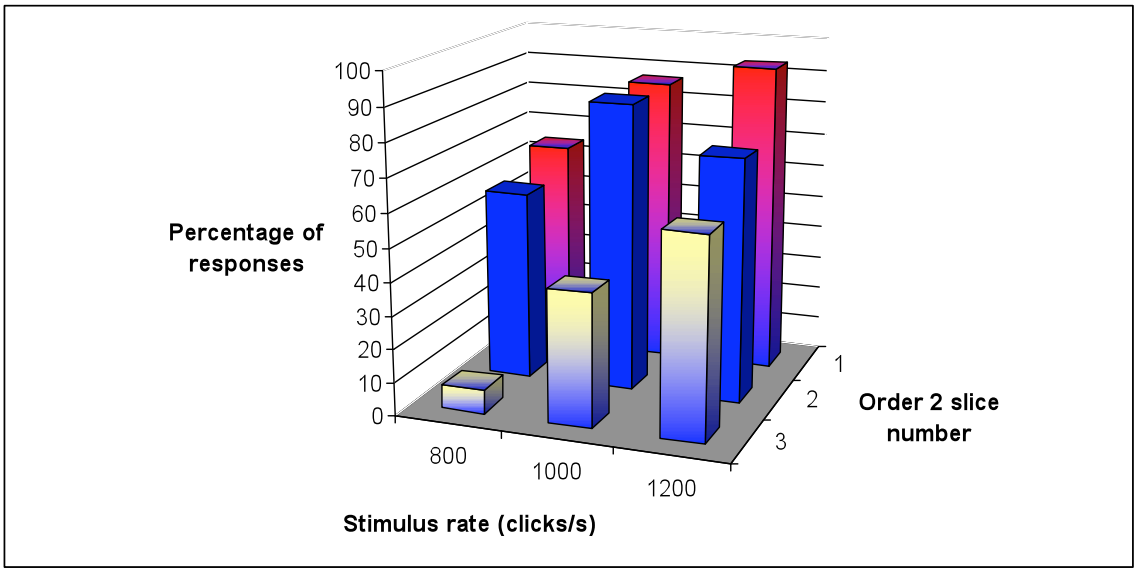


Figure 4.39. *Left ears with absent SOAEs. VSOAE 2nd order responses at stimulus rates tested for the 2-6ms time window (Percentage of responses= those left ear responses with correlation>0.5/ all left ear responses *100((for that particular measure)).*

Therefore, when analysing the effect of rate on ear side, the VSOAE S_{21} has been selected. When analysing the effect of order on ear side the VSOAE S_{21} and S_{31} have been selected. This slice together with VSOAE S_{22} has been selected for the interaction between slice and ear side.

Analysis of the 3rd order slices for both ears revealed the greatest number of responses to be obtained with the VSOAE S_{31}, at a stimulus rate of 1000 clicks/s, as shown in **Figures 4.40 and 4.41**.

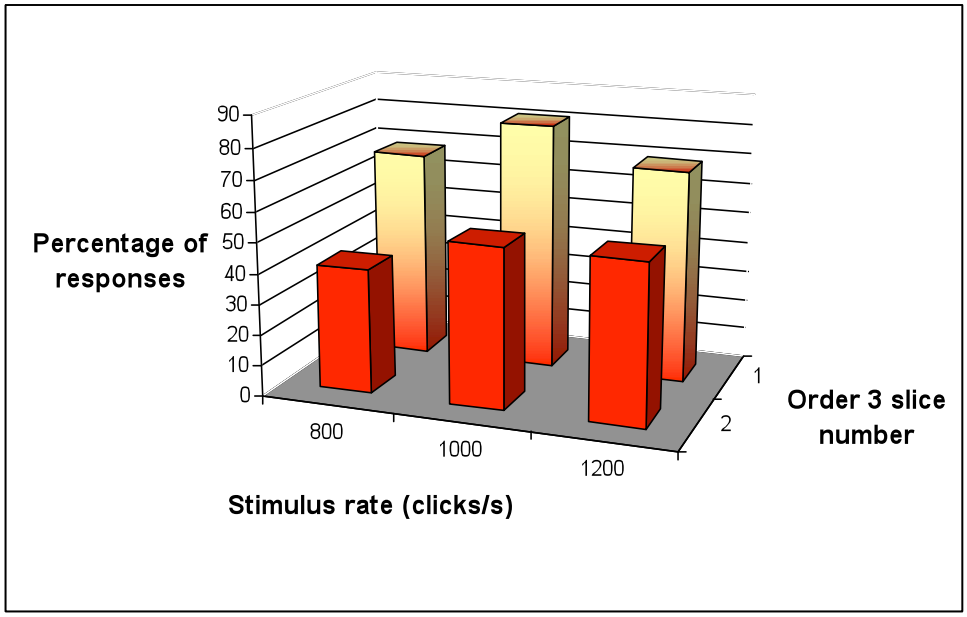


Figure 4.40. *All right ears respectively with VSOAE 3rd order responses at stimulus rates tested for the 2-6ms time window (Percentage of responses= those right ear responses with correlation>0.5/ all right ear responses *100((for that particular measure)).*

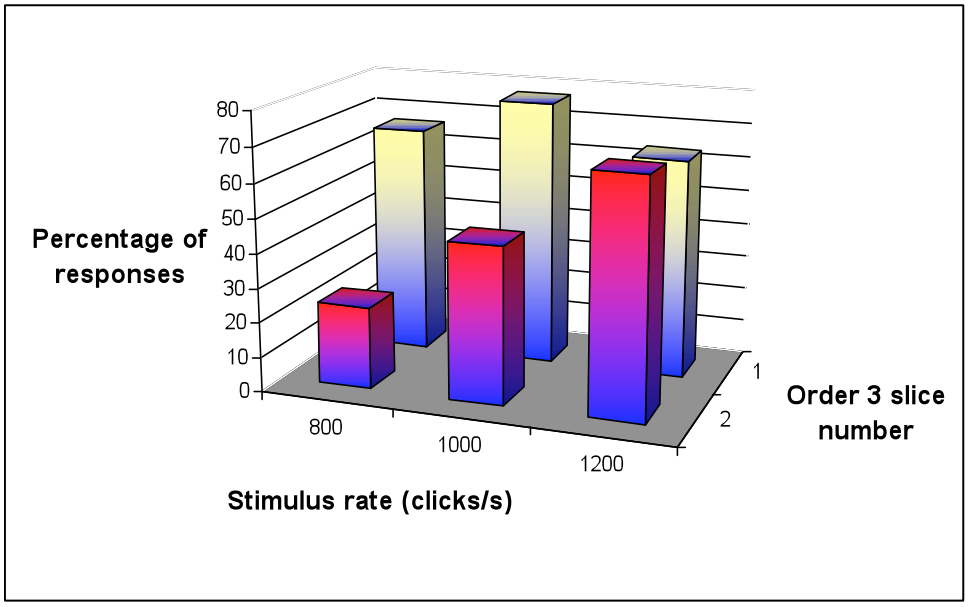


Figure 4.41. *All left ears respectively with VSOAE 3rd order responses at stimulus rates tested for the 2-6ms time window (Percentage of responses= those left ear responses with correlation>0.5/ all left ear responses *100((for that particular measure)).*

When SOAEs were absent once more the most responses were obtained for the VSOAE S_{31}, at a stimulus rate of 1000 clicks/s as portrayed in **Figures 4.42 and 4.43**. However, unexpectedly more responses were obtained for this slice in the SOAE negative ear group compared with all ears (**Figures 4.40 & 4.42**). In our data analysis we have selected the VSOAE S_{31}, at a stimulus rate of 1000 clicks/s, to evaluate the interaction between order and ear.

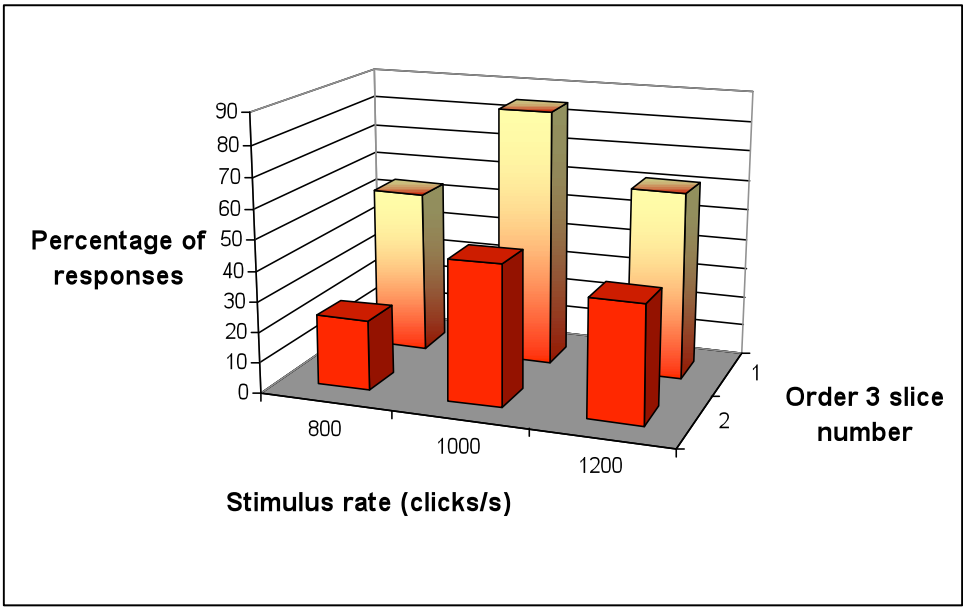


Figure 4.42. *Right ears with absent SOAEs. VSOAE 3rd order responses at stimulus rates tested for the 2-6ms time window (Percentage of responses= those right ear responses with correlation>0.5/ all right ear responses *100((for that particular measure)).*

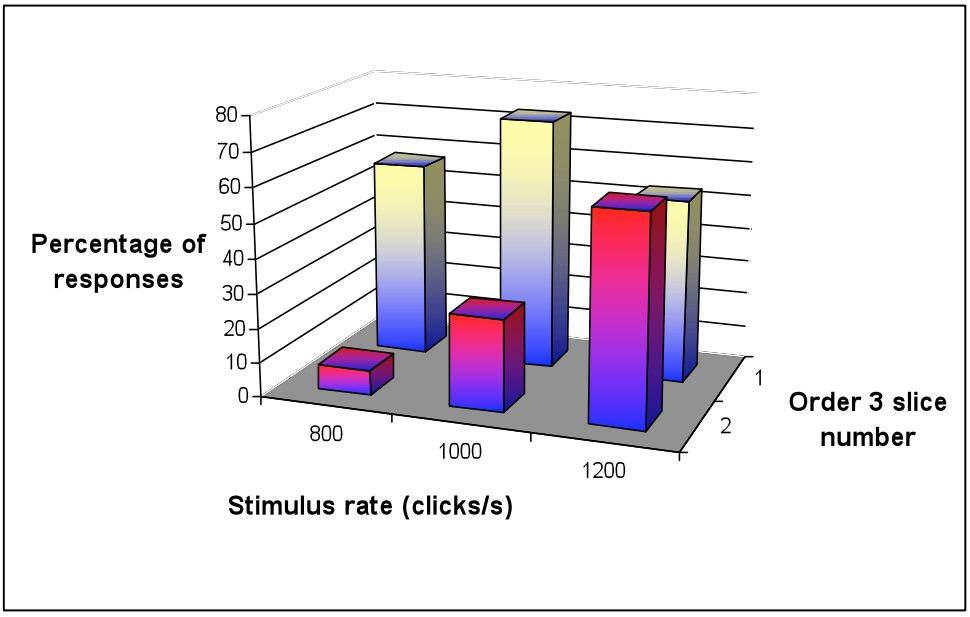


Figure 4.43. *Left ears with absent SOAEs. VSOAE 3rd order responses at stimulus rates tested for the 2-6ms time window (Percentage of responses= those left ear responses with correlation>0.5/ all left ear responses *100((for that particular measure)).*

Following the calculation of the best CEOAEs and VSOAEs to use in our statistical analysis, (the CEOAE at 70 dBpeSPL, 6-17ms time window and VSOAEs S_{21}, S_{22} and S_{31}, obtained at a stimulus rate of 1000 clicks/s), it was necessary to calculate the amplitude of these valid responses in all ears and SOAE-negative ears. In order to evaluate the right/left asymmetry more accurately paired ears with valid responses were selected. Thus the true right/left asymmetry between individuals could be assessed. However, although this is a more accurate method, it did mean fewer responses could be analysed than if assessing the difference between all right ears and all left ears. **Figures 4.44-4.51** depict the root mean square amplitudes obtained for subjects with valid responses in whom both ears were tested.

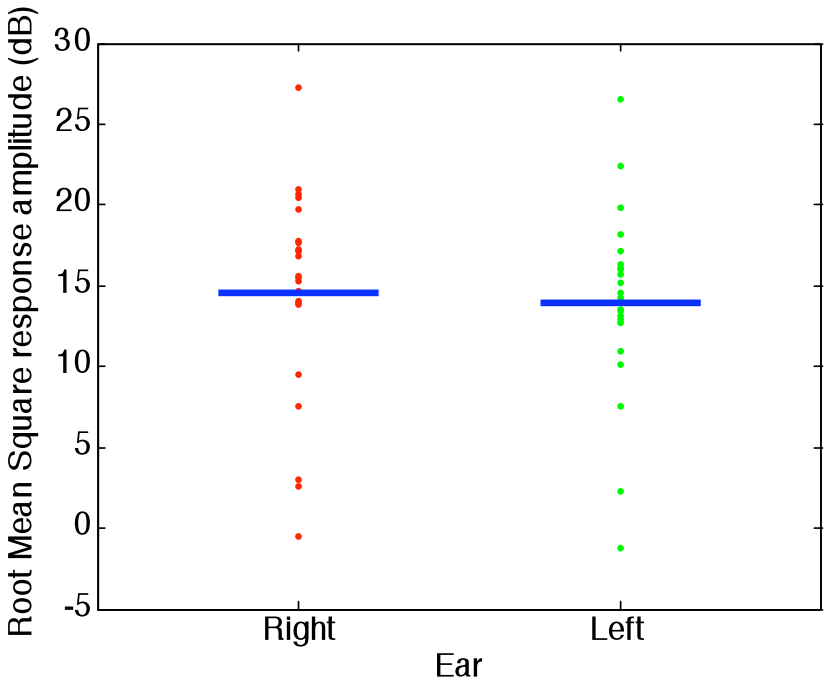


Figure 4.44. *Distribution of the RMS response amplitude for CEOAEs all paired responses (regardless of SOAE status, for both sexes, obtained at conventional rate of 40 clicks/s), for 6-17 ms time window, at a click at stimulus level 70 dBpeSPL, for all responses analysed with correlation >0.5. Bold lines indicate mean.*

CEOAE	Ear	N
	Right	20
	left	20

Table 4.13. *Number of included responses (N) for CEOAE for all paired right and left ears.*

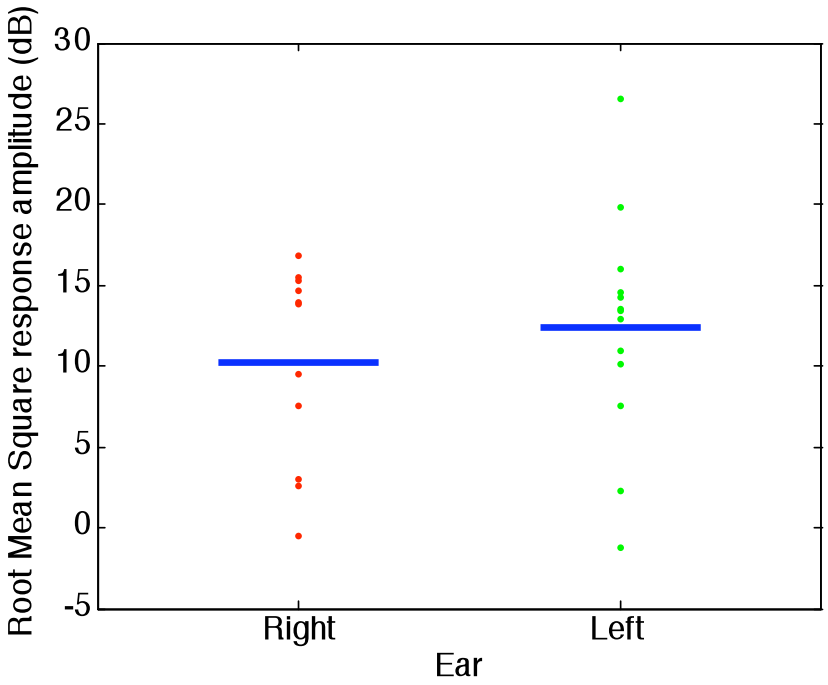


Figure 4.45. *Distribution of the RMS response amplitude for CEOAEs, SOAEs absent, for paired responses, for the 6-17ms time window, at a stimulus level of 70 dBpeSPL, when SOAEs are absent, responses analysed with correlation>0.5. Bold lines indicate mean.*

CEOAE	Ear	N
	Right	12
	left	12

Table 4.14. *Number of included responses (N),for paired responses in ears with absent SOAEs.*

In the case of CEOAEs, in all paired cases (females and males combined) the amplitude of the right ear emission appears to be greater than that of the left ear, as can be seen in **Figure 4.44**. This does not seem to be the case when SOAEs are

absent, however, it must be noted that there are only 12 paired responses in the SOAE negative group, as shown in **Figure 4.45**. The numbers of responses compared were far fewer in the SOAE negative group as demonstrated in **Tables 4.13 & 4.14**. There was no significant difference in the amplitude of the emission between right and left ears, following application of the Bonferroni correction. **Table 4.15** shows the paired samples t-test result for all paired responses and those in SOAE negative ears. However, with so few paired responses in the SOAE-negative group the result must be treated with caution.

CEOAE	Ear	N	t	df	Sig.(2-tailed)
All paired responses	1 2	20 20	1.765	18	0.094
Paired responses in SOAE negative ears	1 2	12 12	3.748	4	0.020

Table 4.15. *Comparison of Paired samples t-test results for interaction between ear and CEOAE, where ear 1=right and ear 2=left.*

The distribution of the right and left ear responses for paired ears was subsequently calculated for the VSOAEs. **Figures 4.46-4.51** show the distribution for the valid paired right and left responses (same subject) for the VSOAEs. **Tables 4.16-4.21** show the numbers of paired ears with valid responses in the groups. The right/left ear difference was not significant for any of the VSOAEs; although for the VSOAE S_{21}, in all valid, paired cases (SOAEs absent and present, with correlations>0.5) the left ear amplitude was shown to be marginally greater than the right ear amplitude and this ear asymmetry approached significance (p= 0.091). However, on application of the Bonferroni correction this result was no longer significant. In addition due to the small numbers of paired responses compared in SOAE absent cases it is difficult to provide any valid interpretation of the effect of ear side. The paired samples T-test results for the VSOAEs are summarised in **Table 4.22**. In contrast to results obtained above with CEOAEs, principally the right ear emission being of greater amplitude than the left ear

emission, albeit not significant, for the VSOAE S_{21}, VSOAE S_{22} and VSOAE S_{31}, the left ear emission appeared to have a greater amplitude than the right ear emission (**Figures 4.46-4.51).**

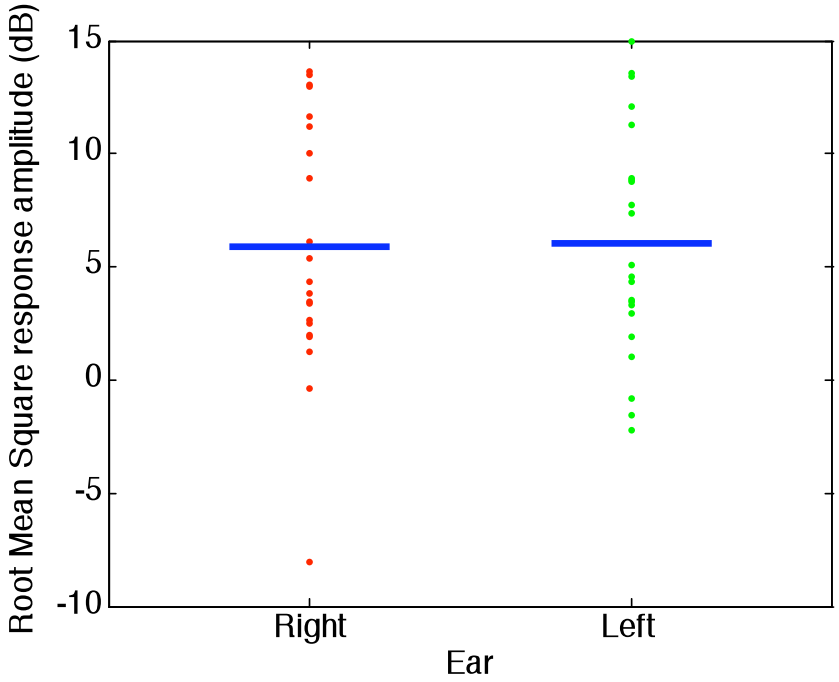


Figure 4.46. *Distribution of the RMS response amplitude for paired responses for the VSOAE S_{21}, (regardless of SOAE status, for both sexes) for all valid responses (corr>0.5), at a click stimulus rate of 1000 clicks/s, 2-6ms time window. Bold lines indicate mean.*

VSOAE S_{21}	**Ear**	**N**
	Right	20
	Left	20

Table 4.16. *Number of included responses (N) for VSOAE S_{21} for all paired right and left ears.*

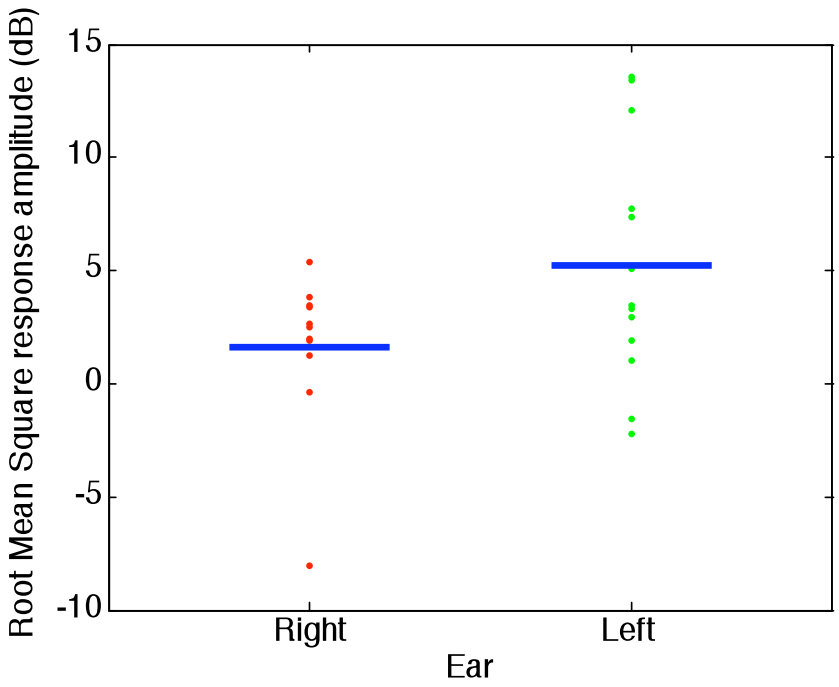


Figure 4.47. *Distribution of the RMS response amplitude for paired responses for the VSOAE S_{21}, when SOAEs are absent, for valid responses (corr>0.5), at a click stimulus rate of 1000 clicks/s, 2-6ms time window. Bold lines indicate mean.*

VSOAE S_{21}	**Ear**	**N**
	Right	11
	Left	11

Table 4.17. *Number of included responses (N) for VSOAE S_{21}, in paired ears with absent SOAEs.*

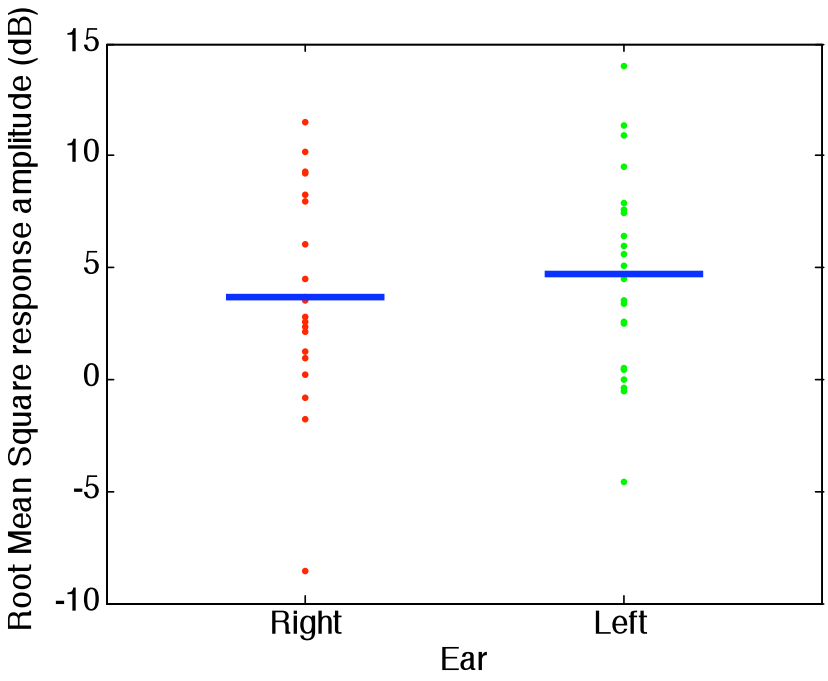


Figure 4.48. *Distribution of the RMS response amplitude for paired responses for the VSOAE S_{22}, (regardless of SOAE status, for both sexes) for all valid responses (corr>0.5), at a click stimulus rate of 1000 clicks/s, 2-6ms time window. Bold lines indicate mean.*

VSOAE S_{22}	**Ear**	**N**
	Right	20
	Left	20

Table 4.18. *Number of included responses (N) for VSOAE S_{22} for all paired right and left ears.*

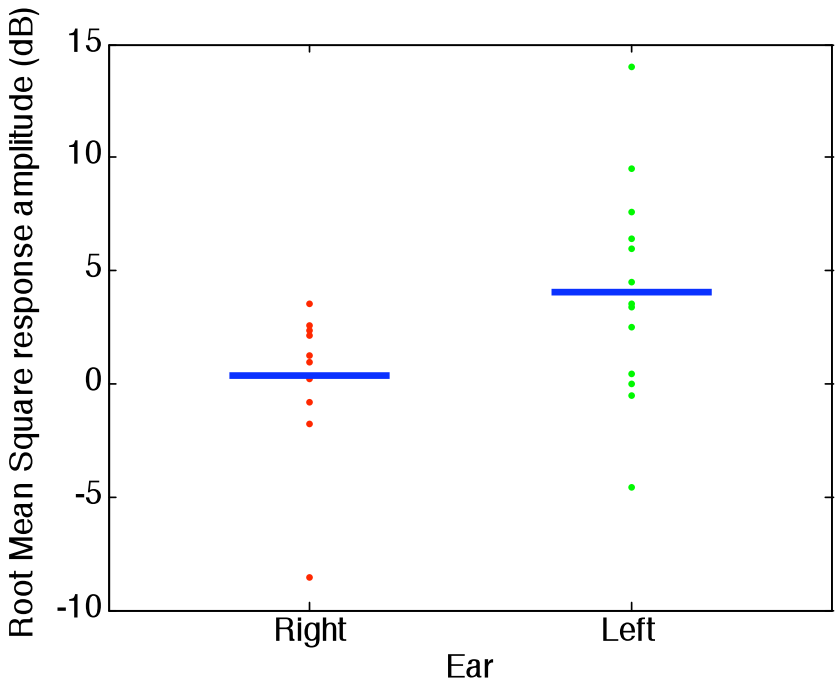


Figure 4.49. *Distribution of the RMS response amplitude for paired responses for the VSOAE S_{22}, when SOAEs are absent, for valid responses (corr>0.5), at a click stimulus rate of 1000 clicks/s, 2-6ms time window. Bold lines indicate mean.*

VSOAE S_{22}	**Ear**	**N**
	Right	12
	Left	12

Table 4.19. *Number of included responses (N) for VSOAE S_{22}, in paired ears with absent SOAEs.*

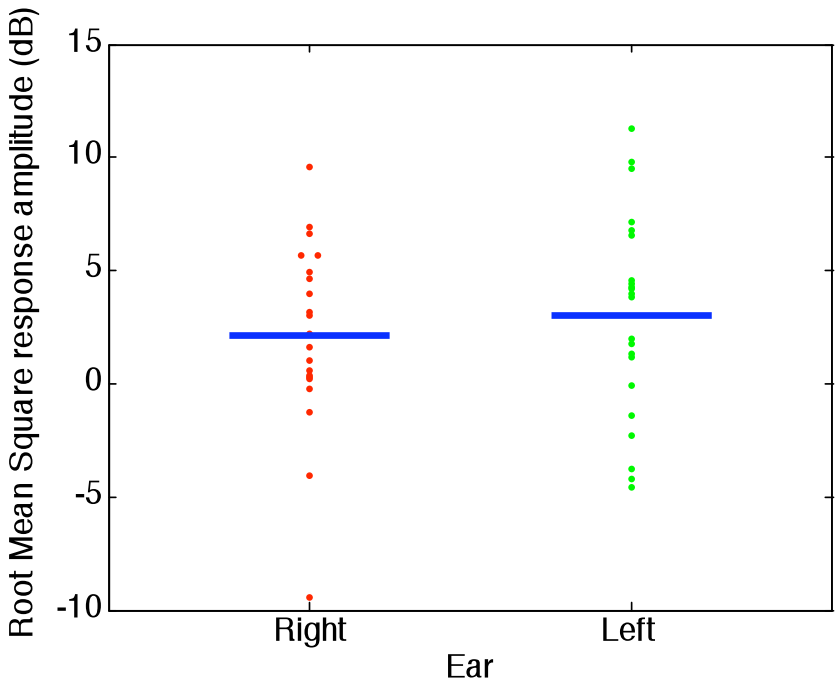


Figure 4.50. *Distribution of the RMS response amplitude for paired responses for the VSOAE S_{31}, (regardless of SOAE status, for both sexes) for all valid responses (corr>0.5), at a click stimulus rate of 1000 clicks/s, 2-6ms time window. Bold lines indicate mean.*

VSOAE S_{31}	**Ear**	**N**
	Right	20
	Left	20

Table 4.20. *Number of included responses (N) for VSOAE S_{31} for all paired right and left ears.*

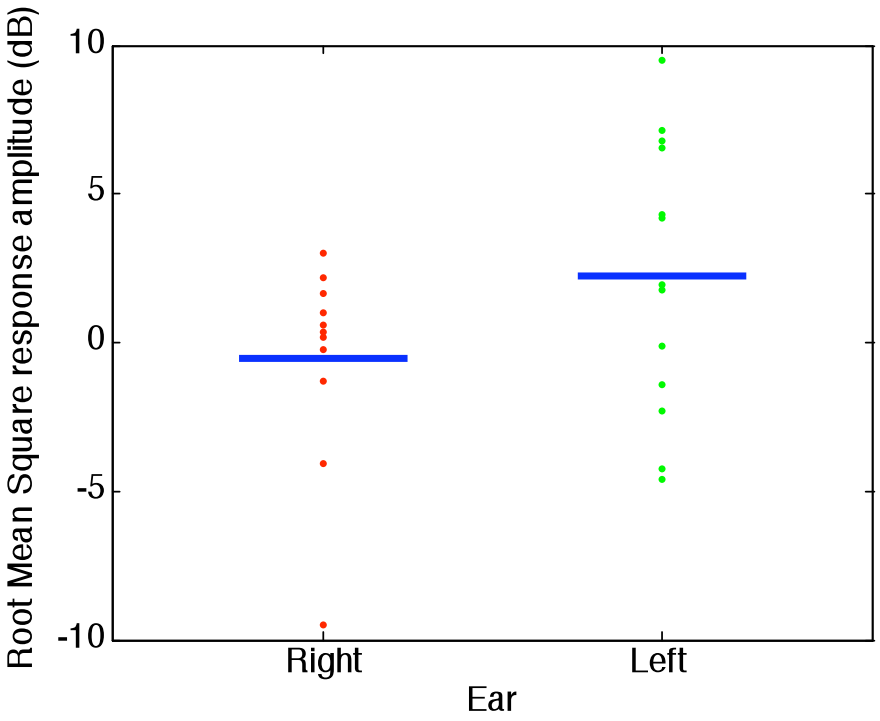


Figure 4.51. *Distribution of the RMS response amplitude for paired responses for the VSOAE S_{31}, when SOAEs are absent, for valid responses (corr>0.5), at a click stimulus rate of 1000 clicks/s, 2-6ms time window. Bold lines indicate mean.*

VSOAE S_{31}	**Ear**	**N**
	Right	11
	Left	11

Table 4.21. *Number of included responses (N) for VSOAE S_{31}, in paired ears with absent SOAEs.*

VSOAE	Condition	Ear	N	t	df	Sig.(2-tailed)
S_{21}	All responses	1 2	20 20	1.815	14	0.091
S_{21}	*Responses in SOAE negative ears*	1 2	11 11	-0.005	3	0.996
S_{22}	All responses	1 2	20 20	0.947	16	0.358
S_{22}	*Responses in SOAE negative ears*	1 2	12 12	1.357	3	0.268
S_{31}	All responses	1 2	20 20	-0.668	11	0.518
S_{31}	*Responses in SOAE negative ears*	1 2	11 11	-0.375	2	0.744

Table 4.22. *Comparison of Paired samples t-test results for interaction between ear and VSOAEs. Ear 1= right, ear 2= left.*

Statistical analysis was undertaken using repeated measures ANOVA to assess the interaction between rate and ear using the VSOAEs S_{21}, obtained at stimulus rates of 800, 1000 and 1200 clicks/s. A significant effect of rate was noted F= 4.357, p=0.01. However, there was no significant interaction between rate and ear F=0.660, p=0.521. **Figure 4.52** shows the main effect of rate.

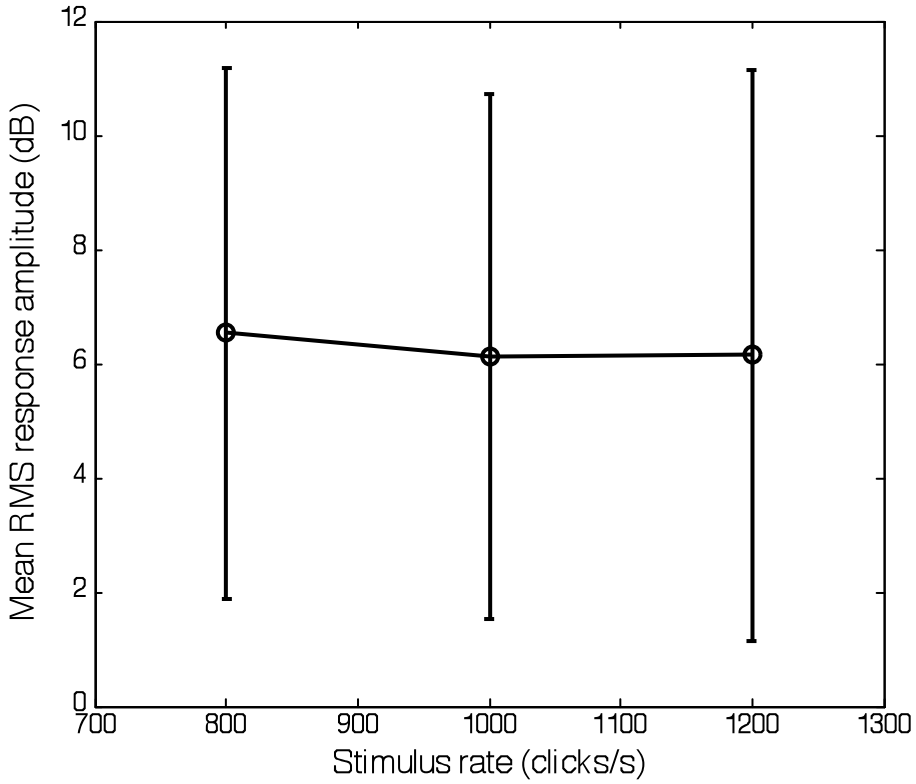


Figure 4.52. *The variation of the mean response amplitude with the stimulus rate, for VSOAE 2nd order (slice 1) responses for paired right ears and left ears combined, in those responses where SOAEs were present and absent, for the 2-6 ms time window. Error bars are shown.*

Overall, the amplitude of the response decreases with an increase in stimulus rate as shown in **Figure 4.52** above. The decrease in the amplitude of the response is greater between stimulus rates 800 and 1000 clicks/s, in comparison with the decrease in amplitude between stimulus rates of 1000 and 1200 clicks/s.

Repeated measures ANOVA was again used to evaluate the interaction between order and ear for the VSOAE S_{21} and VSOAE S_{31} at a stimulus rate of 1000 clicks/s. There was a highly significant main effect of order, F= 66.642 and p< 0.0005, this being a greater response amplitude with the second order slice 1 compared with the third order slice 1 response amplitude (**Figure 4.53**). There was no significant interaction between order and ear, F= 1.807 and p= 0.188.

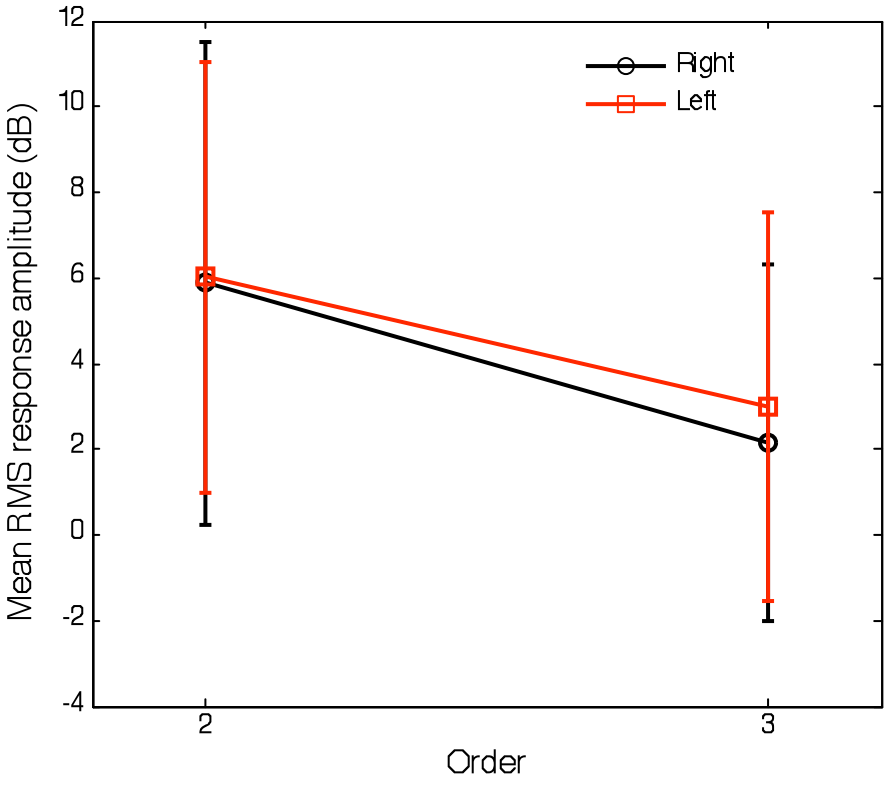


Figure 4.53. *The variation of the mean of the response amplitude with the order, for VSOAE 2nd order and 3rd order (S_{21} and S_{31}) responses for right ears and left ears respectively, in those responses where SOAEs were present and absent, for the 2-6 ms time window. Error bars are shown.*

Finally, the interaction between slice and ear was investigated using general linear modeling, repeated measures ANOVA. The main effect of slice was found to be highly significant (F= 23.824, p< 0.0005) with the VSOAE S_{21} producing a response of greater amplitude than the VSOAE S_{22}, as depicted in **Figure 4.54**. The interaction between slice and ear was not significant, F= 0.484, p= 0.491.

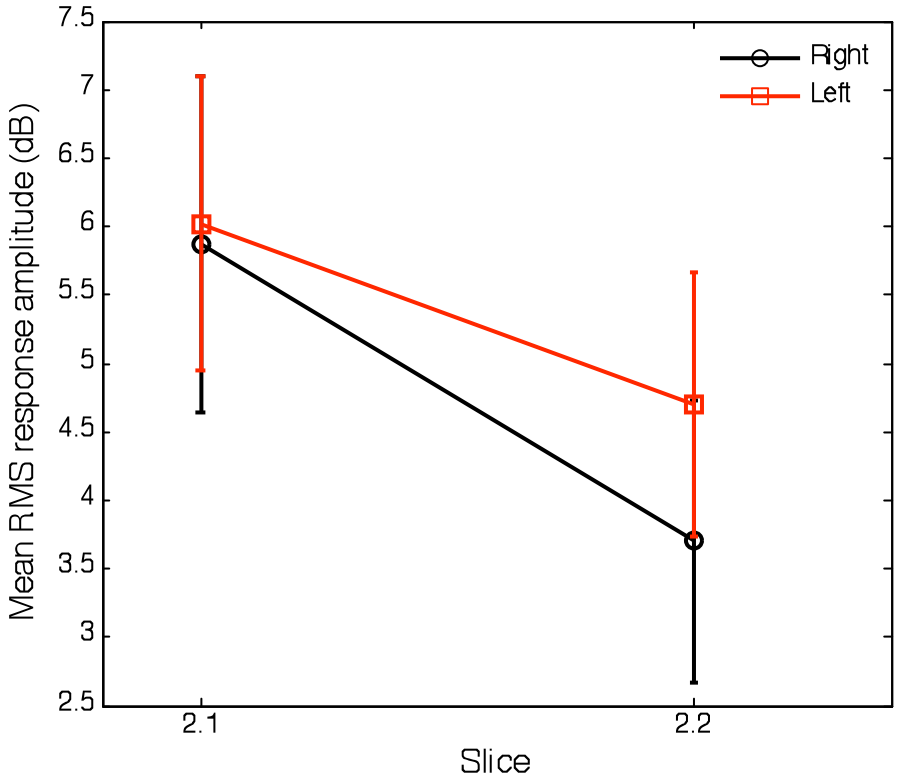


Figure 4.54. *The variation of the mean of the response amplitude with slice, for VSOAE 2nd S_{21} and S_{22} responses for right ears and left ears respectively, in those responses where SOAEs were present and absent, for the 2-6 ms time window. Error bars are shown.*

4.4.4) The effect of sex and side on CEOAEs and VSOAEs

We have shown in sections 4.4.2 and 4.4.3 that the CEOAE and VSOAE slices with the most valid responses are the CEOAE at 70 dBpeSPL, 6-17ms time window and VSOAE S_{21}, S_{22} and S_{31}, at a stimulus rate of 1000 clicks/s respectively. The aim of this analysis was to investigate the differences in the amplitude of these responses between female right and left ears, and male right and left ears. Twenty pairs of ears were tested, 14 of these pairs were female and only six male. On more detailed analysis in ears with valid responses, too few responses were obtained in those with absent SOAEs, and for the VSOAE S_{31}. Therefore, statistical analyses could not be undertaken to accurately reflect the effect; these results are not included as the sample size is too small to deduce any valid result.

Initially the results were analysed for paired female ears with well correlated responses. It was possible to analyse responses, from 14 right ears and 14 left ears for the CEOAE (**Table 4.23**). Females were shown to emit CEOAEs of greater amplitude from their right ears than from their left ears, as shown in **Figure 4.55**. This female right ear increased amplitude response was not found to be significant, on paired samples T-test, p= 0.172.

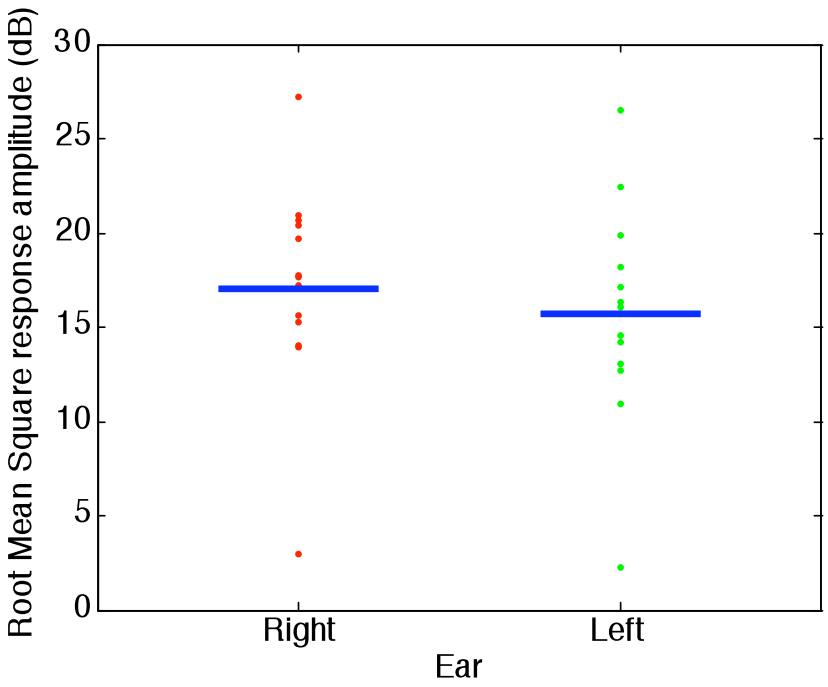


Figure 4.55. *Distribution of the RMS response amplitude for the CEOAE, for all valid female paired responses (corr>0.5). Bold lines indicate mean.*

CEOAE	Ear	N
	Right	14
	Left	14

Table 4.23. *Number of included responses (N) for CEOAE for all* ***female*** *paired right and left ears.*

In the case of the VSOAE S_{21}, looking at paired female ears; emissions of greater amplitude were recorded from female right ears. This result approached significance, on paired samples T-test p= 0.066. However, on application of the Bonferroni correction, the result was no longer significant. The effect of sex and side on the VSOAE S_{21} in females, and the number of responses analysed, are shown in **Figure 4.56** and **Table 4.24** respectively.

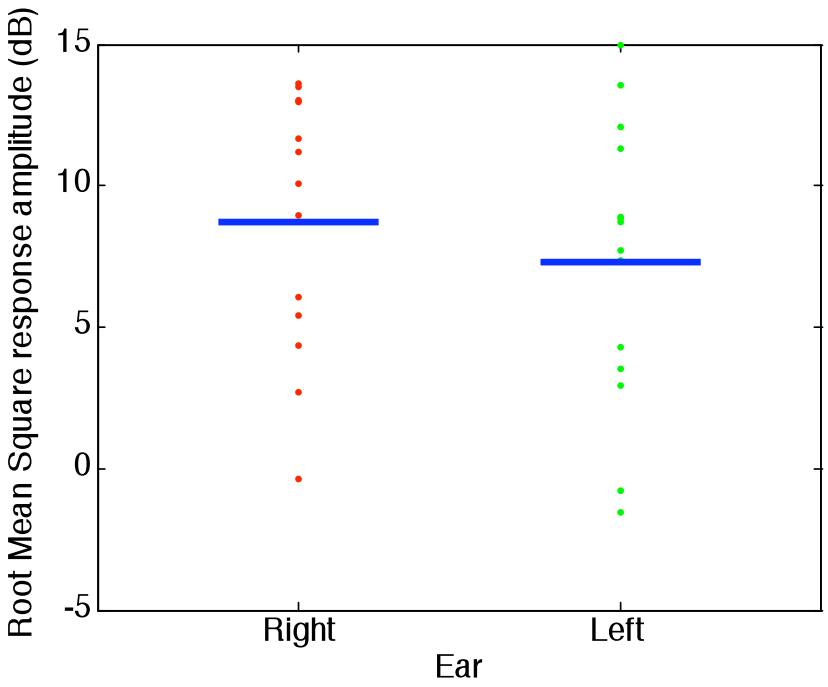


Figure 4.56. *Distribution of the RMS response amplitude for the VSOAE $S_{21,}$ for all valid female paired responses (corr>0.5), at a click stimulus rate of 1000 clicks/s, 2-6ms time window. Bold lines indicate mean.*

VSOAE S_{21}	**Ear**	**N**
	Right	13
	Left	13

Table 4.24. *Number of included responses (N) for VSOAE S_{21} for all* ***female*** *paired right and left ears.*

On paired samples T-test for all valid female responses, no significant result was obtained for the VSOAE S_{22} (p= 0.419). Emissions of slightly greater amplitude were obtained from female left ears when compared with female right ears. This is shown in **Figure 4.57**, and the numbers of responses included can be seen in **Table 4.25**.

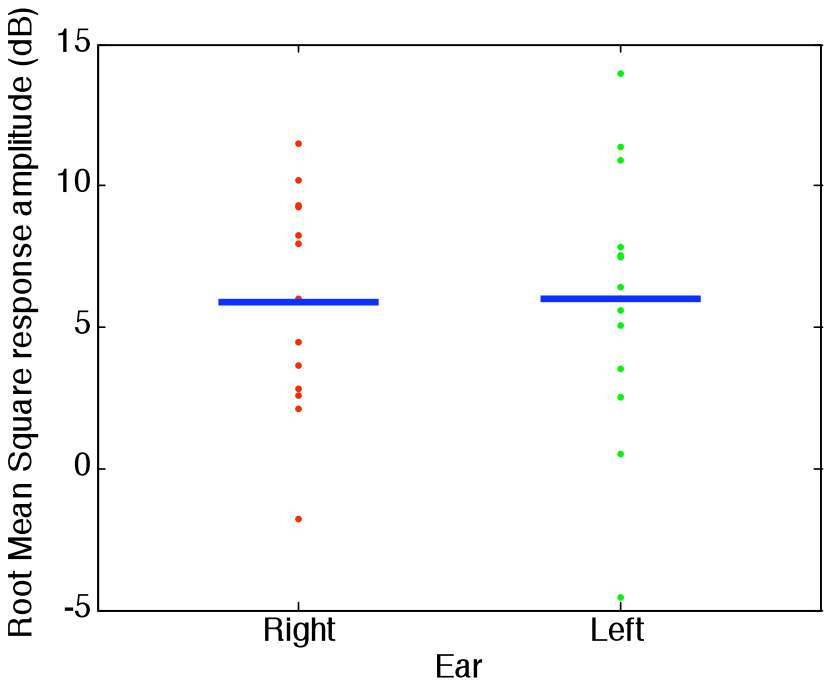


Figure 4.57. *Distribution of the RMS response amplitude for the VSOAE $S_{22,}$ for all valid female paired responses (corr>0.5), at a click stimulus rate of 1000 clicks/s, 2-6ms time window. Bold lines indicate mean.*

VSOAE S_{22}	**Ear**	**N**
	Right	13
	Left	13

Table 4.25. *Number of included responses (N) for VSOAE S_{22} for all* ***female*** *paired right and left ears.*

For male subjects all responses were analysed, and only slices with enough valid responses for a statistically valid result were analysed. When analysing the CEOAE paired male responses (six right and six left ears, as can be seen in **Table 4.26**), no significant male right/left asymmetry was demonstrated, although male right ears appeared to have emissions of greater amplitude than male left ears (p= 0.376) (**Figure 4.58**).

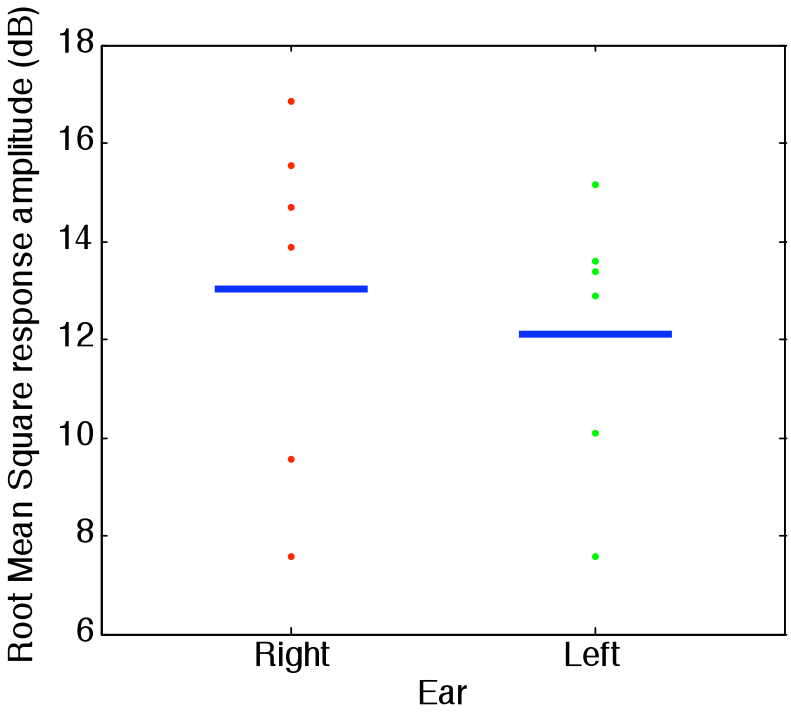


Figure 4.58. *Distribution of the RMS response amplitude for the CEOAE, for all valid male paired responses (corr>0.5). Bold lines indicate mean.*

CEOAE	Ear	N
	Right	6
	Left	6

Table 4.26. *Number of included responses (N) for CEOAE for all* ***male*** *paired right and left ears.*

In the case of the VSOAE S_{21}, in paired male responses no significant ear difference was demonstrated (p= 0.322), although male right ears appeared to have responses of greater amplitude than male left ears. However, there were only six paired responses analysed in this case. These results are depicted in **Figure 4.59** and the numbers of responses included shown in **Table 4.27**.

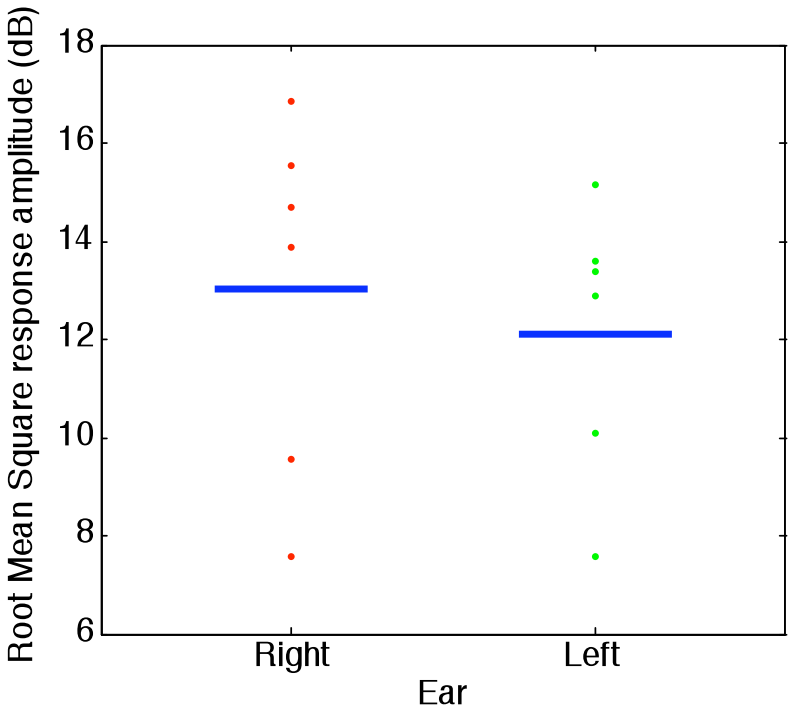


Figure 4.59. *Distribution of the RMS response amplitude for the VSOAE $S_{21,}$ for all valid male paired responses (corr>0.5). Bold lines indicate mean.*

VSOAE S_{21}	**Ear**	**N**
	Right	6
	Left	6

Table 4.27. *Number of included responses (N) for VSOAE S_{21} for all* ***male*** *paired right and left ears.*

The results for the right/left ear asymmetry for paired male responses in the case of the VSOAE S_{22} were not found to be significant on paired samples T-test (p= 0.719). **Figure 4.60** shows the amplitude of the responses obtained from left ears to be greater than those obtained from right ears. **Table 4.28** shows the number of responses analysed.

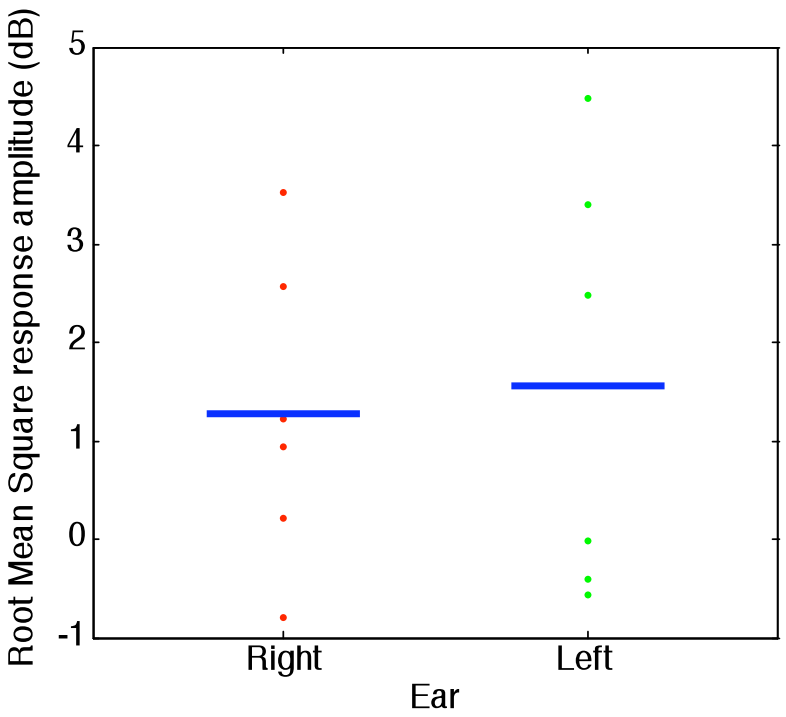


Figure 4.60. *Distribution of the RMS response amplitude for the VSOAE $S_{22,}$ for all valid male paired responses (corr>0.5). Bold lines indicate mean.*

VSOAE S_{22}	**Ear**	**N**
	Right	6
	Left	6

Table 4.28. *Number of included responses (N) for VSOAE S_{22} for all **male** paired right and left ears.*

For males as with females, the results could only be analysed in all paired responses, as the sample size was already small before applying any exclusions (e.g. those without SOAEs). In addition it was not possible to analyse the third order response as too few valid responses were obtained (3 paired responses).

The interaction between rate and ear, and also slice and ear was investigated for paired female and paired male responses separately. It was not possible to study the effect of ear and order due to too few responses being obtained for paired responses for the VSOAE S_{31}. On repeated measures ANOVA no significant effect of rate (F=

0.548, p= 0.588), or interaction between rate and ear (F= 1.358, p= 0.282) was seen, for paired female responses. However, in paired male ears a significant main effect of rate was demonstrated (F= 5.365, p= 0.039), but there was no interaction between rate and ear (F= 0.006, p= 0.994). It can be seen in **Figure 4.61** that the amplitude of the emission from both right and left ears from paired male responses, decreases with an increase in the stimulus rate**.**

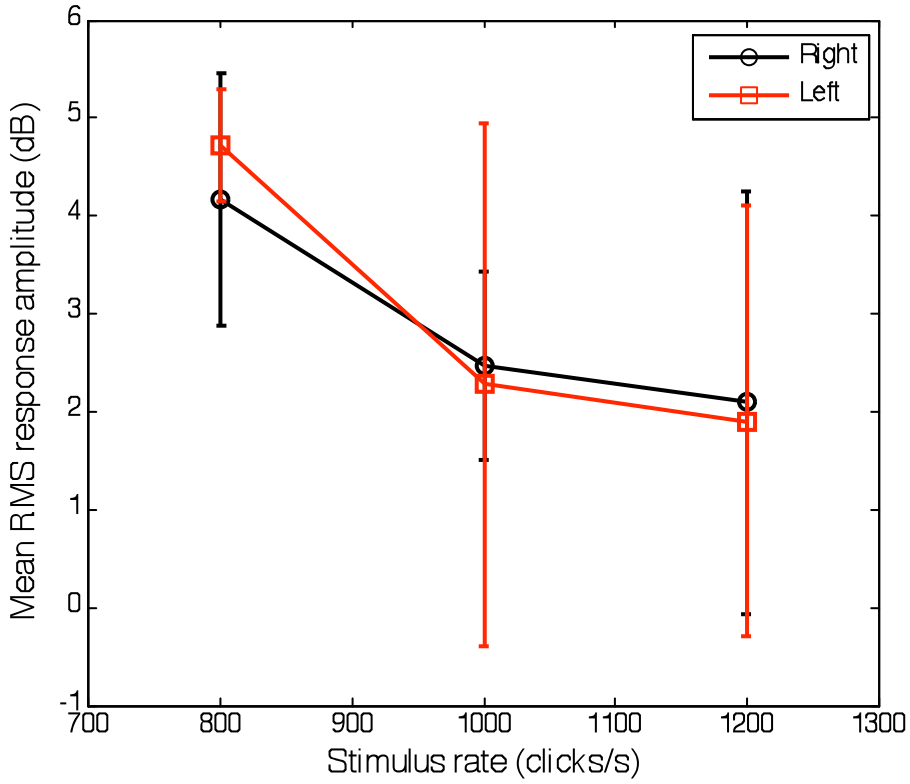


Figure 4.61. *The variation of mean of the response amplitude with the rate, for VSOAE S_{21} at stimulus rates of 800, 1000 and 1200 clicks/s responses for paired male right ears and left ears respectively, in those responses where SOAEs were present and absent, for the 2-6 ms time window. Error bars are shown.*

There was a significant main effect of slice for paired female responses (F= 15.269, p= 0.001), but no interaction between slice and ear (F= 1.542, p= 0.226). The amplitude of the response was less for the VSOAE S_{22} than for the VSOAE S_{21} (**Figure 4.62**).

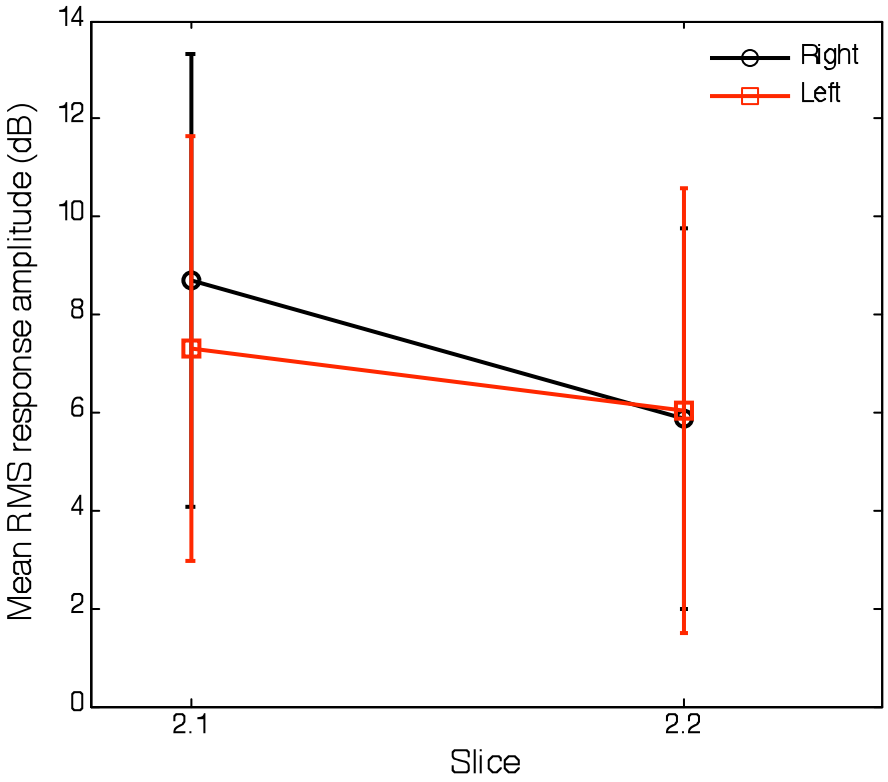


Figure 4.62. *The variation of the mean of the response amplitude with slice, for VSOAE 2nd S_{21} and S_{22} responses for paired female right ears and left ears respectively, in those responses where SOAEs were present and absent, for the 2-6 ms time window. Error bars are shown.*

There was also a significant main effect of slice for paired male responses (F= 8.637, p= 0.017), but no interaction between ear and slice (F= 0.745, p= 0.410). This effect was the same as that obtained in all cases for ears with slice with a decreased response amplitude for the VSOAE S_{22} compared with the VSOAE S_{21} (**Figure 4.63**).

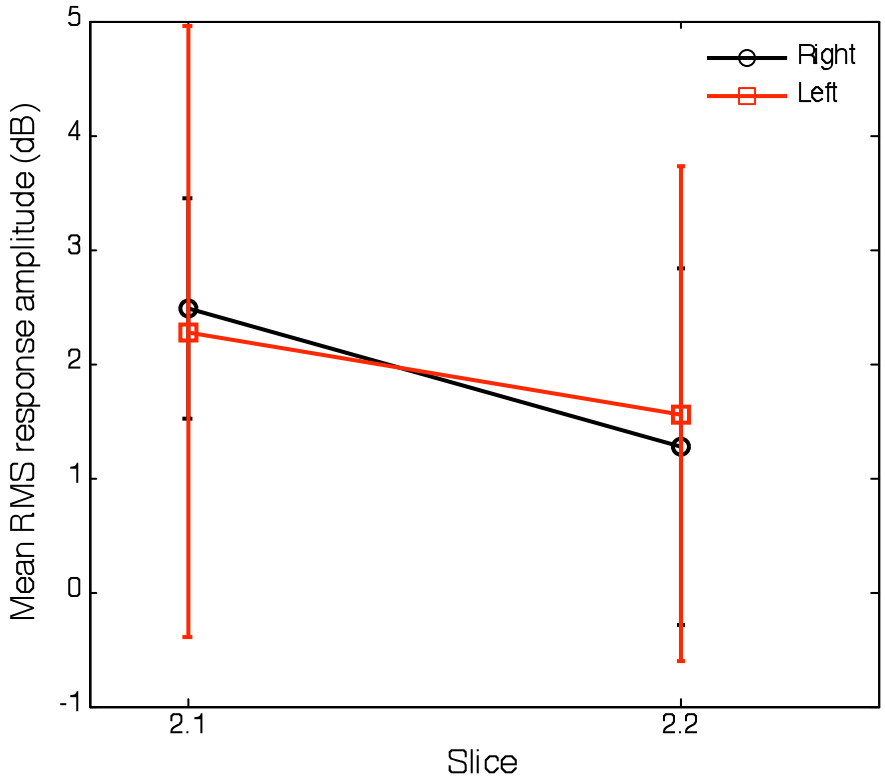


Figure 4.63. *The variation of the mean of the response amplitude with slice, for VSOAE 2nd S_{21} and S_{22} responses for paired male right and left ears respectively, in those responses where SOAEs were present and absent, for the 2-6 ms time window. Error bars are shown.*

4.5) Discussion of the effect of sex, side and SOAEs on VSOAEs

4.5.1) The effect of SOAES on CEOAEs and VSOAEs

SOAEs were recorded in 66.7% of the ears tested. Probst et al (1990) in their review of otoacoustic emissions, found that overall in 11 studies conducted involving surveys of SOAEs in various human populations, that SOAEs could be detected in about one third of ears of normally hearing individuals.[14] However, they proposed that the incidence of SOAEs may depend on the sensitivity of the recording system. There is much acoustic background noise in the ear canal; these are mainly low frequency noises associated with breathing, blood flow, muscle contractions and temporo-mandibular joint noises etc. Therefore as no stimulation is needed to record SOAEs, a microphone with a high sensitivity, low noise floor and the ability to detect the smallest possible measuring volume, so that sound pressures of small amplitude SOAEs are enhanced must be used.[14] The Probst review also mentioned the presence of multiple SOAEs from single ears. More recently Kuroda (2007) in his clinical investigation on SOAEs in 447 ears (268 females, 179 males, 222 left ears and 225 right ears), in infants (33 ears) and school children and adults (414 ears), age range 0- 75 years (mean 30.8 years) found an incidence of SOAEs in the whole of normally hearing ears was approximately 38%.[142] A similar result of an incidence of 40% was reported by Bilger et al (1990).[143] Currently with better instrumentation the incidence of SOAEs has been found to be between 60 and 70%, in normally hearing adults, in agreement with the results obtained in our study.[10, 144, 145]

The incidence of SOAEs and number of SOAEs per ear were found to be higher in the subjects of age 50 years or less, in those with a hearing level of not more than 30 dB, in the right ear, and in females by Kuroda (2007).[142] When SOAEs are found they usually occur in the 1000- to 2000-Hz region; amplitudes are between -5 and 15 dB SPL. Some individuals have multifrequency SOAEs over a broader frequency range.[146] OAEs typically are bilateral rather than unilateral, thus if there is an SOAE in one ear, there is an increased chance of finding one in the other ear (though not at

the same frequency).[146] If unilateral, they are more likely to be present in the right rather than in the left ear and occur more often in females than in males, as was shown in our study.[146]

An important point that needs to be considered is whether the effect attributed to SOAEs, primarily an increased number of responses in their presence and responses of greater amplitude in their presence is indeed due to them, or an effect of sex. **Table 4.0** showed that the majority of female ears (which one would expect to produce larger emissions) had SOAEs whereas the majority of male ears were SOAE negative. Thus a possibility is that the effect ascribed to the SOAE is actually an effect of sex.

As one would expect the greatest number of evoked responses were obtained for the CEOAE at the highest stimulus level tested 70 dBpeSPL. In their study of transient evoked otoacoustic emissions recorded using maximum length sequences as a function of stimulus rate and level, Hine and Thornton (1997), demonstrated an increase in the mean root mean square amplitude with an increase in click level, which was slightly more noticeable at lower levels of stimulation and click rates.[105] At low levels transient evoked otoacoustic emission amplitude increases almost linearly with stimulus level. However, at higher levels the (above about 55 dBpeSPL) the middle and latter parts of the emission saturate, thus the nonlinear I/O function.[147] Hence the greatest number of responses are obtained at this plateau. There were a greater number of valid responses obtained for the CEOAEs when SOAEs were present, and also the amplitude of the CEOAE was increased in the presence of SOAEs. These effects were found to be highly significant. It has been suggested that delayed evoked, synchronous evoked and spontaneous otoacoustic emissions are very closely related to each other, and that as such result from the same source within the cochlea.[148] SOAEs exist continuously, whereas other emissions can be seen in response to short sound impulses and show up after a delay of about 10 ms, such responses are termed delayed evoked emissions.[148] Synchronous evoked emissions are a third type of emission that appear as a

response to a continuous tone, have the same frequency as the stimulating tone and are synchronous with the stimulating tone, from which the emission has to be extracted by special methods.[148] This correlation between delayed and spontaneous otoacoustic emissions may be used to explain the greater number of good quality responses obtained and the increased amplitude of the response for the CEOAE when SOAEs were present.

In this study responses selected were those with waveform correlations of >0.5, which is equivalent to a signal to noise ratio of 1, or SNR= 0dB. This was performed as this enables one to ensure the quality of the responses selected, which is especially important in the smaller sized samples. In addition if small responses with low SNRs were included it would be analogous to comparing noise from ears rather than signal. Furthermore, as the estimator for the Root Mean Square amplitude was contaminated by noise, the choice was made to estimate responses with good SNRs so that contamination by noise would not have affected the response significantly. The subjects included in the experiments were of normal hearing and healthy individuals, thus overall provided good responses. However, it has been suggested that as small responses are more affected by noise than large ones, if the ‘’unbiased estimator’’ is not used, there is a tendency to overestimate the true amplitude, when the correlation coefficient is low. For example, if the repeat waveform correlation is 0.5, the amplitude obtained directly from the waveform tends to be 3 dB too high (due to the presence of noise). This could potentially have an impact on some of the results although unlikely due to the subject population and the good SNR selected as mentioned above.

For the VSOAEs the slices with the greatest number of valid responses for comparing rate were the VSOAE S_{21}, at all stimulus rates tested (800, 1000 and 1200 clicks/s). In the comparison of order the best slices were the VSOAE S_{21} and VSOAE S_{31}. When comparing slice, the slices with the most valid responses were the VSOAE S_{21} and VSOAE S_{22}. In this current study the VSOAE S_{21} had the highest amplitude, followed by the VSOAE S_{22}, and finally the VSOAE S_{31}. The VSOAEs of order 1 (the

MLSOAEs) are of greater amplitude than those of orders 2 and 3, typically by a factor of 5.[80] In prior studies the largest number of qualifying responses for the higher order (nonlinear) VSOAEs has been the second followed by the third, then fourth and fifth orders.[80] For the second order upwards the greatest number of valid responses have been found with slice one, followed by slice two, followed by slice three and so on.[80] The stimulus rate has been shown to influence the VSOAE greatly.[80, 86] Low amplitude VSOAEs have been observed for stimulus rates of 4282 and 5000 clicks/s for all orders, slice numbers and stimulus levels; and stimulus rates of 1000 or 1500 clicks/s tested in earlier studies have been shown to give the largest number of 'good' slice waveforms both for the second and third orders.[80, 86] Thus in the comparison of order and slice a stimulus rate of a 1000 clicks/s was used in the current study which tallies well with previous studies, where similar rates showed the greatest number of valid responses.

The effect of the presence of SOAEs on the VSOAE response amplitude, this being a higher response amplitude when SOAEs were recorded, was found to be highly significant in the cases of the VSOAE S_{21}, VSOAE S_{22} and VSOAE S_{31}. This is likely to be the case as the higher order VSOAEs (2^{nd} order and above) are nonlinear temporal interaction components, and it is thought that SOAEs arise from nonlinear processes in the cochlea. Moreover, morphological indications have been found showing that SOAE generation is likely to be related to local irregularities of outer hair cell distribution along the organ of Corti.[149] Therefore, this result reflects the fact that both the SOAE and VSOAE are nonlinear and are likely to arise from a similar cochlea mechanism. Indeed prior studies have shown that the sources responsible for SOAE generation also contributed to the generation of stimulus following OAEs and TEOAEs.[149] Indeed a preliminary study at the Institute of Hearing Research several years ago showed that SOAE-positive ears produced VSOAEs of greater amplitude than SOAE-negative ears, and therefore that SOAE activity reflects the nonlinearity of the cochlea. However, the VSOAE amplitude and the SOAE amplitude did not correlate.[150]

There was a significant main effect of rate for the VSOAE S_{21} response amplitude, in the presence of SOAEs. The amplitude was greater in the presence of SOAEs, and with an increasing stimulus rate from 800 to 1200 clicks/s, the amplitude of the response decreased. The main decrease occurred between stimulus rates of 800 and 1000 clicks/s. In the absence of SOAEs the amplitude of the VSOAE S_{21}, decreased from a stimulus rate of 800 to 1200 clicks/s. Although for all ears (those with and without SOAEs) the main effect of rate was a decrease in the response amplitude with increasing rate. Slaven et al (2003) illustrated that as the stimulus rate increases from very low rates, the amplitudes increase, reach a maximum and then decrease with further increase in rate. Between 1500 and 3000 clicks/s, the VSOAE reduced in amplitude with stimulus rate becoming scarcely measurable at 4282 and 5000 clicks/s.[80] Thus it is likely that as the slope of the decrease is greatest between 800 and 1000 clicks/s, the reduction in amplitude is likely to occur between these points. In the absence of SOAEs there were far fewer responses, although more than included in other studies, therefore the effect may not be valid due to an insufficient number of responses. Alternatively SOAE negative ears produce weaker VSOAEs therefore the response is less pronounced, and possibly the reduction in the amplitude of the response commences at a lower stimulus rate, namely between 800 and 1000 clicks/s.

The significant interaction between VSOAE order and the presence of SOAEs for the VSOAE S_{21} and S_{31} was expected for the reasons described earlier. Namely, the VSOAE amplitude is greater in the presence of SOAEs, as they both arise from nonlinear properties; and the amplitude of the second order slice is greater than that of the third consistent with previous findings by this group.[80]

The relationship between slice and VSOAE amplitude was found to be significant for the VSOAE S_{21} and $S_{22,}$ the amplitude being greater for the first slice, in agreement with Slaven et al (2003). The interaction between VSOAE slice and the presence of SOAEs was not found to be significant. This maybe as a result of too small a sample

size and a small difference between the two, or indeed no significant effect of VSOAE slice on SOAE.

4.5.2) The effect of sex on CEOAEs and VSOAEs

SOAEs were found in 83.3% of female ears and 33.3% of male ears consistent with the findings of other studies described earlier.[142, 143] This finding has been accounted for by assuming that the tendency to exhibit emissions is inherited, perhaps as a sex-linked trait.[143]

The CEOAE response for the 6-17 ms time window, at 70 dB was selected for statistical analysis as this demonstrated the greatest number of good quality responses, as described above. Once again the most 'good' responses were obtained for the VSOAE S_{21}, S_{22} and S_{31}, at the stimulus rates tested, the reasons for this have been discussed above.

The CEOAE amplitude (conventional rate, linear response) following the Bonferroni correction was shown to approach significance ($p= 0.007$) with female responses being of greater amplitude than male responses, in keeping with the results obtained in chapter 3. The result was not significant when data for only those females and males in which SOAEs were absent was analysed. This is possibly as a result of too small a sample size (9 females and 14 males) to show the difference that would be expected. Another possible explanation for no female/male asymmetry in SOAE-negative ears is that SOAEs may be a consequence of generally strong emissions. Females without SOAEs may be similar to males without SOAEs, both just having weak emissions. The proposed theories for female subjects having emissions of greater amplitude than male subjects have been fully discussed in **Chapter 3**.

The VSOAE S_{21} and VSOAE S_{22} response amplitudes were found to be significantly higher in females than in males when all cases with valid responses were included. The greater emission amplitude in females was not found to be significant in the case

of the VSOAE S_{31} and in all the chosen slices when the SOAEs were absent. This is assumed to be a consequence of the majority of ears having SOAEs, thereby resulting in a significantly decreased number of responses analysed when excluding those with SOAE positive ears, or as mentioned above as a result of weaker responses in ears without SOAEs. In the case of the VSOAE S_{31} as the response is smaller than for the second order, a smaller difference is likely to occur, and therefore obtaining a significant difference is unlikely. Alternatively, although unlikely, there is no significant female/male asymmetry for the third order. The explanations for this female male difference have been discussed in **Chapter 3**.

There was a significant interaction between the VSOAE S_{21} response amplitude, stimulus rate and sex, with female subjects having responses of greater amplitudes than males at all rates tested. The decline in the amplitude of the response (gradient of the slope) was greater in males than in females. This female preponderance is expected, and the change with rate tallies with the results obtained showing the relationship between VSOAE second order response amplitude and rate found earlier in this series of experiments.

The VSOAE S_{21} and S_{31} response amplitudes were significantly related to the sex of the subject and the order. Females had emissions of greater amplitude for both orders, but the decline in response amplitude from the second to third orders was greater in males. This may occur as with fewer male responses this effect is more pronounced, or indeed in males there is a greater decline in the amplitude of the response due to a difference in the auditory system.

There was no significant interaction between slice and sex, probably as a result of the small difference between the two successive slices VSOAE S_{21} and S_{22} such that the number of responses being analysed was too small to detect this difference. There may, although unlikely, be no female/male difference between these two slices, as prior results have been obtained for linear responses and conventional OAEs demonstrating the increased female response amplitude.

4.5.3) The effect of side on CEOAEs and VSOAEs

The responses used in the analysis of the effect of side on the CEOAE and VSOAE were those with the largest number of valid responses (those with waveform correlations>0.5). The CEOAE, 6-17 ms time window, at 70 dB (at 40 click/s) and VSOAEs S_{21}, S_{22} and S_{31}, at all stimulus rates tested (800, 1000, 1200 clicks/s) when looking at the effect of rate for the S_{21} and at a stimulus rate of 1000 clicks/s for the S_{22} and S_{31}. In this part of the study the results from 20 pairs of ears were analysed. There were not as many subjects recruited in this study as in earlier experiments (Chapter 3) this was partly due to problems recruiting subjects, especially males in a centre some distance away from the main hospital and also student buildings and accommodation. Also the results were obtained in addition to those required to investigate the relationship between nonlinear OAEs (Chapter 5), and based on previous studies and it was suggested that 45 ears would be required.

Right ears were not shown to emit responses of significantly greater amplitude than left ears in all cases for the CEOAEs. However the amplitude of the emission from right ears was graphically shown to be greater than that from left ears, for all paired responses regardless of SOAE status. The result may have been insignificant due to the small sample size and earlier studies have shown this difference may be very small. The greater amplitude emission from the right ear is in agreement with the findings of other studies, which also found this phenomenon to occur with transient evoked otoacoustic emissions.[51, 54, 120, 121, 136, 151] This right ear predominance also seems to be associated with a preponderance of SOAEs.[52, 57, 152] Thus one might expect to find an increased right/left ear difference when SOAEs are included, and a smaller difference for SOAE negative ears. The reasons why the right ear emission may be bigger than the left have been described in **Chapter 3**.

When analysing the results for the VSOAEs left ear responses were found to be larger than right ear responses overall. None of the results were significant. However, it must be noted the number of responses analysed was relatively small; for VSOAE

S_{21}, 20 pairs were analysed, for VSOAE $S_{22,}$ 20 pairs were analysed and for VSOAE S_{31}, 20 pairs were analysed. In SOAE negative ears the valid paired ear responses analysed was too small to interpret any result; for VSOAE S_{21}, 11 pairs were analysed, for VSOAE $S_{22,}$ 12 pairs were analysed and for VSOAE S_{31}, 11 pairs were analysed. Another factor that needs to be considered is that these were the data for only paired ears, thus should be more accurate, as the difference is the right/left asymmetry between same subject ears; therefore, the greater number of female subjects should not influence the right/left ear difference. There is limited evidence from previous studies that no ear difference does exist. Cassidy et al (2001) in their study of transient evoked otoacoustic emissions, in 350 newborns; 170 males and 180 females, found no significant difference due to ear.[55] Right ear mean response was 12.37 dB and left ear mean response was marginally greater at 12.88 dB, the responses were statistically similar.[55] Kowalska et al (1994), in their study of evoked otoacoustic emissions in 44 subjects showed insignificant differences in responses from right and left ears.[153]

The order of testing effect in otoacoustic emissions and its consequences for sex and ear differences in neonates has previously been described by this group, and was published following the first series of experiments in Chapter 3, and has been referred to in the discussion of the results in that chapter.[108] Briefly, this study, in agreement with the results obtained here demonstrated that females gave emissions of greater amplitude than males (1.2 dB greater response). The measured right/left ear difference was enhanced when the right ear was tested first, but was diminished when the left ear was tested first.[108] Following the discovery of this phenomenon, in this set of investigations the ear to be tested first was alternated between subjects, to eliminate this effect. Therefore, as mentioned earlier, it is likely that the paired data will reflect the true relationship between side and the CEOAE and VSOAEs. Therefore, in this part of the study the subject numbers were either too small, the responses weak from right ears in this population, or indeed that no significant right/left asymmetry exists as discussed above.

As has been previously shown, there was a significant interaction between the VSOAE response amplitude and rate, the VSOAE response amplitude and order, and the VSOAE response amplitude and slices. There were no significant interactions between these factors and the ear side. Overall there was a decrease in the VSOAE S_{21} with an increase in rate (**Figure 4.52**). This is in agreement with earlier findings which have shown that the VSOAE response amplitude tends to decrease with the stimulus rate above a certain stimulus level. Alternatively, it may be that the decrease is above a stimulus rate of 1000 clicks/s. The findings of a decrease in VSOAE response amplitude with order for the VSOAE S_{21} and VSOAE S_{31} and slice for the VSOAE S_{21} and VSOAE S_{22} have been explained earlier.

4.5.4) The effect of sex and side on CEOAEs and VSOAEs

The results for paired female and male responses were analysed, and the numbers of responses included are shown in the respective tables. 20 pairs of ears were tested 14 of which were female and 6 of which were male. This obviously impacts the results, but a trend is demonstrated for what one would expect to find, albeit not significant. It was only possible to use the data for responses where SOAEs were both present and absent, due to too small a sample size in those with SOAE negative ears. Focusing on paired female responses, right ears were shown to have bigger responses than left ears for the VSOAE S_{21} and CEOAE, consistent with findings obtained with conventional evoked otoacoustic emissions. None of these differences was significant. The maximum number of paired ears tested for females was 14. However, the greater female right ear response amplitude supports both the finding of a right ear increased response amplitude and also the order of testing phenomenon.[51, 54, 108, 121]. However, it is likely once again the sample size is too small.

Only 15 male ears, 6 pairs were included in the study; this was due to difficulties with male subject recruitment, and failure of several males to pass the entry criteria of the study. Thus, when looking at paired male data few valid responses could be included.

Despite this, a larger CEOAE right ear response amplitude was obtained. When analysing the VSOAE S_{21}, male right ears were found to have responses of greater amplitude than male left ears, again too few responses were included (6 pairs). The relevance of these differences for paired male responses is difficult to determine due to the small sample size. Prior to the experiment we estimated the need to recruit 45 ears. Unfortunately, due to the entry criteria, it was difficult to balance for sex and side. In addition as the VSOAEs are thought to more accurately reflect changes in cochlear function, as shown in changes with mild hearing loss, a thought was that perhaps the right/left ear asymmetry, if originating at a cochlear level would be more evident in the case of VSOAEs.[86] Thus a smaller sample size may be sufficient, as only the responses of 12 ears of 9 individuals were required to show a difference in previous studies with VSOAEs.[86]

There was no significant effect of rate for the VSOAE S_{21} response amplitude in paired female responses, although in males there was a significant main effect of rate for the VSOAE S_{21}. This main effect of stimulus rate as discussed previously was a decreased response amplitude as the stimulus rate increased from 800 to 1200 clicks/s. This group has shown that there is a decrease in the response amplitude with higher stimulus rates.[93] The effect of order could not be assessed due to the reduced sample size, although one could postulate based on earlier results obtained when investigating the female/male asymmetry that the higher the order (3), the smaller the response amplitude (i.e. response amplitude is higher for the second order slice). There was a significant main effect of slice number and VSOAE response amplitude for both paired female and male responses respectively. No interaction was found to exist between the VSOAE response amplitude, slice and ear for the paired female and male responses respectively. The effect of VSOAE response amplitude for the S_{21} and S_{22} slices was a decline in the response amplitude with the latter slice, as has been found in our prior results in this current study. These results are also consistent and provide further evidence in support of those obtained by Slaven et al (2003).[80]

In conclusion overall females were shown to have responses of greater amplitude than males. Focusing on paired responses, female subjects were found to have CEOAE and $VSOAE_{21}$ of greater amplitude from their right ears than left ears. In male subjects the sample size was small, so it is not possible to provide a valid interpretation for this result.

CHAPTER 5
RESULTS 3: THE RELATIONSHIP OF VSOAES TO EXISTING NONLINEAR OAE MEASURES

5.1) Introduction

The mammalian cochlear response is nonlinear; an increase in the magnitude of stimulation does not always produce a proportional increase in the velocity or displacement of basilar membrane vibration.[79] As such otoacoustic emissions display nonlinear phenomena. In the amplitude domain the input-output (I/O) functions display nonlinear compressive characteristics, Distortion product otoacoustic emissions (DPOAEs) provide an example of nonlinearity in the frequency domain, using the MLS technique it has been shown that nonlinear temporal interaction waveforms termed VSOAEs can be recorded and SOAEs may arise from nonlinear processes within the cochlea.[100] Therefore the aim of this series of experiments was to investigate the relationship between the temporal nonlinear interaction components, VSOAEs and these other measures of nonlinearity individually and to investigate the relationship with them all. The theory behind this was that the measures of nonlinearity should be related to one another.

5.2) Design of study and protocol

The study design and protocol has previously been described in **Section 4.2**, where SOAE measurement has been described, and a more detailed description of the methods is provided in **Chapter 2**.

After ensuring the subject was of normal hearing status, each subject underwent the routine outlined below:

6. SOAE testing
7. DPOAE testing
8. Repeat SOAE testing
9. Volterra Kernel testing
10. I/O testing

For the collection of the DPOAEs, the same in house designed system used to collect the SOAEs was used again but on the DPOAE setting. Preamplifier gain was set at 40 dB. The pure tone stimuli were presented at fixed levels of 73 dBHL for f1 and 67 dBHL for f2. A sweep between 750 Hz and 4 kHz was undertaken. The mean value of the DPOAE was calculated from this. A repeat sweep was performed.

VSOAES were measured using an in house designed system, and obtained using MLS order 12. Recalibration of the equipment and data collection for the VSOAEs has been described in **Section 4.2** and also detailed in **Chapter 2**.

In order to record the measurements required for the I/O function, the same in house designed system as that used to measure the VSOAEs was used. These responses were recorded at the conventional rate of 40 clicks/s. Stimulus levels of 40. 50, 60 and 70 dBpeSPL respectively were presented, in that order for the first run and in reverse order i.e. 70, 60, 50 and 40 dB respectively for the repeat run. One thousand reconstructions were obtained at each level.

5.3) Analysis procedure

The SOAEs files, four for each subject were all examined for artefacts. They were then imported into Microsoft excel. The average magnitude of the SOAE for the four runs at each frequency was calculated. A graph was created to demonstrate the variation of the average magnitude of the response of the spontaneous otoacoustic emission on the y axis with the frequency on the x axis as shown in **Chapter 4,** section **4.3**. The average magnitude of the spontaneous emission was then calculated from the base to the tip of the peak. If there was more than one emission this was recorded. Emissions of magnitude ~3dB or greater were recorded as valid responses. If no emission was present this was also recorded. The results for each subject were then imported into an SPSS file for data analysis.

Each DPOAE file was examined and the DP gram checked for artifacts. The files were then imported into Microsoft Excel individually and the necessary data extracted. The DPOAE were analysed in octave bands centred at 1, 2 and 4 kHz, and the results analysed for the repeat runs. Only bins with good signal to noise ratio (SNR) >6 dB were included. This was performed by applying a programme written in Matlab (by Professor ARD Thornton) to the data. The data were then imported into SPSS.

In order to analyse the VSOAEs several computer programmes were used. The VSOAE waveforms were first deconvolved from the MLS using a programme written in Matlab by Professor ARD Thornton. Statistical analysis was then performed using SPSS and Excel. The individual VSOAEs were deconvolved from the raw responses to MLS. The 1st order slice (the MLSOAE) corresponds to the 'linear' CEOAE (obtained at 40 clicks/s). The waveforms for the CEOAEs and VSOAEs of every ear were visually inspected to check the waveform lengths, where they started and the waveform correlation. An acceptable waveform correlation was >0.5. **Figure 5.0** shows examples of waveforms of the CEOAEs recorded at different stimulus levels. The amplitudes of the waveforms increase as the stimulus level increases, with more noticeable effects seen in the early time frame (6-9ms). **Figures 5.1 and 5.2** show the amplitude of the waveforms at for both the VSOAE S_2 and S_3 slices. The amplitude of the VSOAE response decreases with increasing slice number and is most noticeable in the 2-6ms time frame. **Figure 5.3** shows an example of the amplitude of the VSOAE S_{21} at the different stimulus rates which were close to one another (800/s, 1000/s and 1200/s).

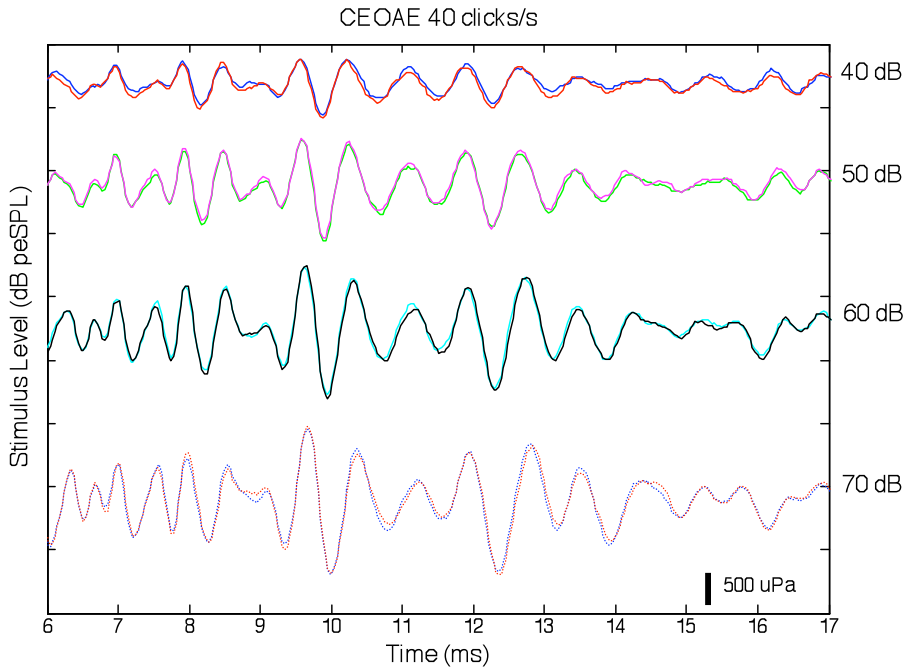


Figure 5.0. *Examples of waveforms of the CEOAEs recorded at different stimulus levels. The amplitudes of the waveforms increase as the stimulus level increases, with more noticeable effects seen in the early time frame (6-9ms).*

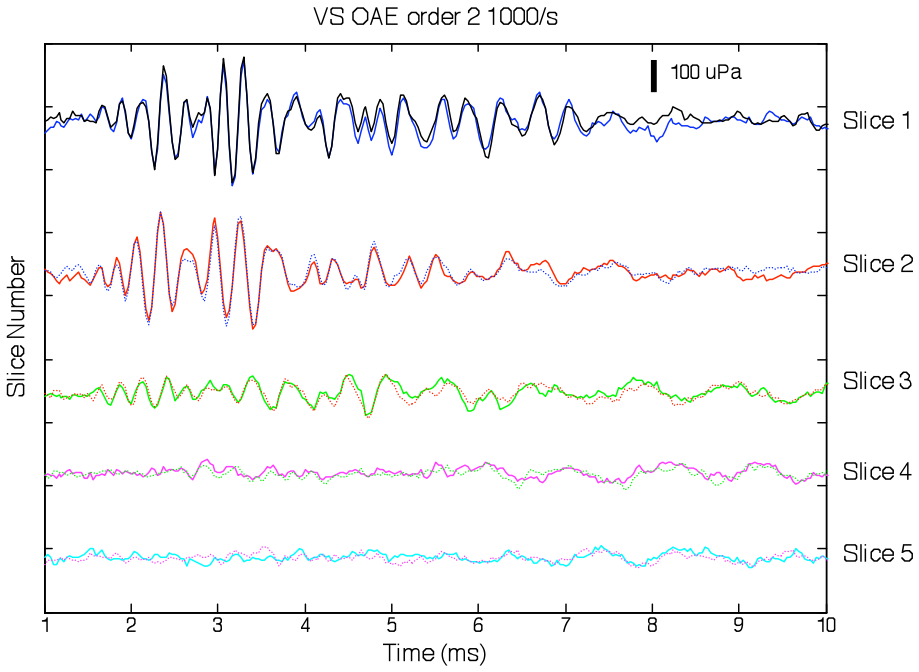


Figure 5.1. *Example of VSOAE second order slices (for a female left ear), obtained at a stimulus rate of 1000 clicks/s. The amplitude of the response decreases with increasing slice number. The main part of the response can be seen to occur in the 2-8 ms time interval (although this depends on the rate, order and slice number).*

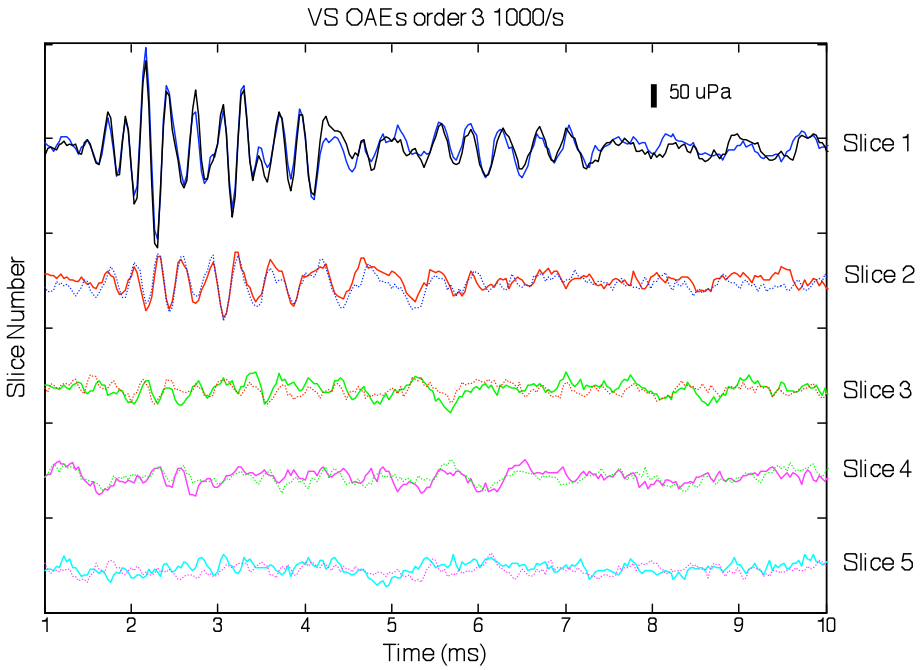


Figure 5.2. *Examples of VSOAE third order slices (for a female right ear), obtained at a stimulus rate of 1000 clicks/s. The amplitude of the response decreases with increasing slice number. The main part of the response can be seen to occur in the 2-8 ms time interval (although this depends on the rate, order and slice number).*

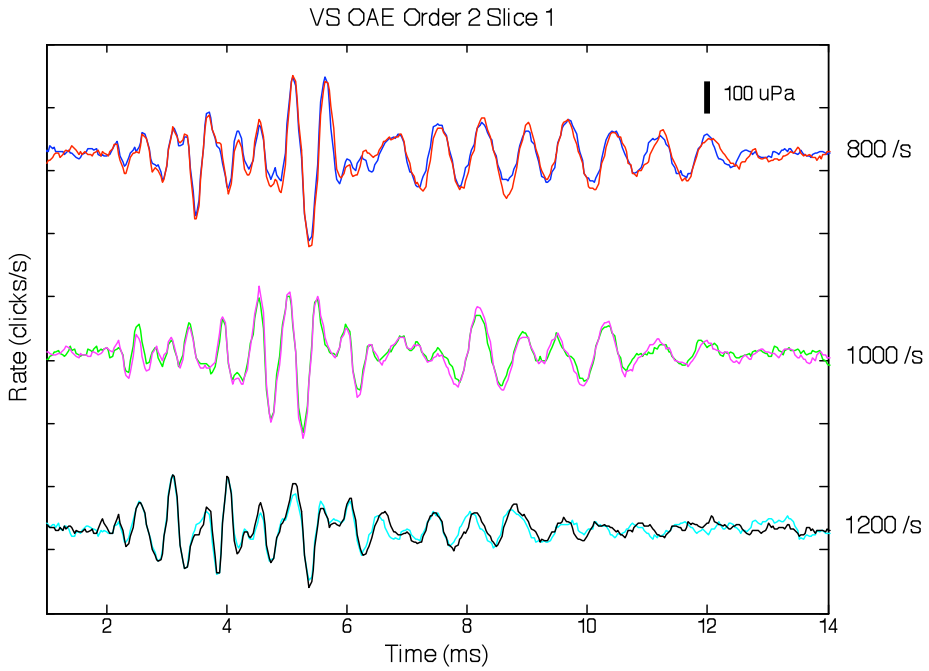


Figure 5.3. *Examples of VSOAE S_{21} (for a female right ear), for all stimulus rates tested. The amplitude of the response is similar in each case as the rates selected for the experiment were close to one another (i.e. 800/s, 1000/s & 1200/s). The main part of the response can be seen to occur in the 2-8 ms time interval (although this depends on the rate, order and slice number).*

The analysis procedure for the CEOAE and VSOAE has been described in **Section 4.3.** The time windows selected for the CEOAE were 0-3ms, 6-9ms, 9-13ms, 13-17ms and 6-17ms. The data analysis was performed for the 6-17ms and 9-13ms time epochs, which have been used in past publications.[100] As there is no stimulus artifact present in the VSOAE and the waveforms are shorter, and occur earlier, different time frames were compared for the VSOAE: 2-6ms, 6-10ms, 2-8ms, 8-14ms, and 2-14ms were chosen. The root mean squared (RMS) amplitudes of the waveforms were calculated for each response. For the calculation of the RMS and the selection on time windows please refer to **Section 4.3**. In short, the cross correlations between the slices were calculated for the 2-6 ms time window and these results were used for the data analysis of the VSOAEs. .

The I/O function was examined by calculating the slope of the I/O function for all ear responses, at a conventional stimulus rate of 40 clicks/s. The I/O function was calculated for each ear by determining the interaction of the root mean square response amplitude of the CEOAE (conventional response) with the level tested between 50 and 70 dBpeSPL. A regression line was then fitted to all the points and the I/O slope function calculated for each ear. If the I/O function was linear then the slope would be 1 dB/dB, however as the I/O function becomes more compressive the slope decreases therefore providing a measure of nonlinearity.[86]

As in the previous chapter the distribution of the responses for the specific entity being analysed were checked for normality using distribution curves, and following this the one sample Kolmogorov-Smirnov test if there was any uncertainty using the former method. As the use of multiple statistical tests may result in significant results by chance, the Bonferroni correction was applied.

The data were analysed using Matlab, SPSS and Microsoft Excel.

5.4) Results

5.4.1) The relationship of VSOAE amplitude with SOAE amplitude

There was a normal distribution for the CEOAE amplitude (obtained at the conventional rate 40 clicks/s) in the 9-13ms time window at both 60 and 70 dBpeSPL respectively. This is shown in **figures 5.4** and **5.5**. The 9-13ms time window was used as this contains the most prominent portion of the TEOAE and this has also proved to be the case with MLSOAE (see chapter 3).

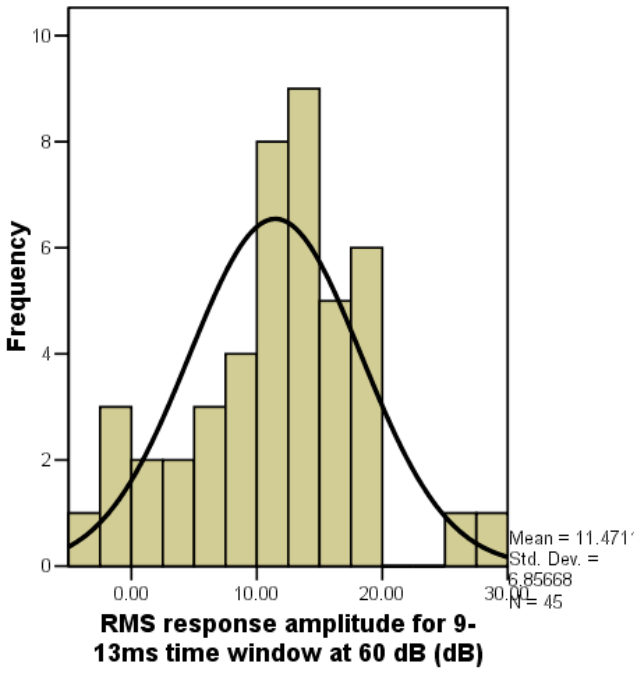


Figure 5.4

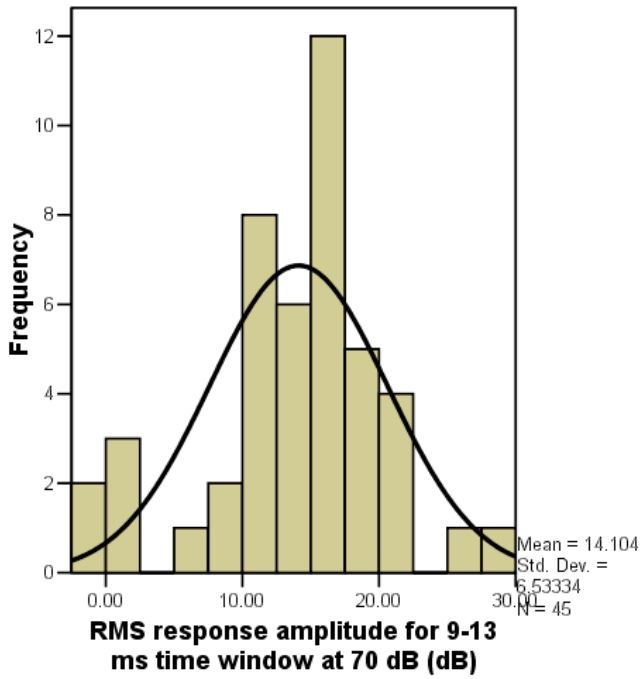


Figure 5.5

Figures 5.4 and 5.5. *Distribution of RMS response amplitudes for all valid responses (waveform correlation>0.5) for CEOAEs, 9-13 time window at 60 dBpeSPL (**figure 5.4**) and 70 dBpeSPL (**figure 5.5**) respectively.*

The association between the amplitude of the CEOAE and the amplitude of the SOAE was found to be significant, in the 9-13ms time window at both 60 (df= 1, 28, F= 7.770, p=0.009) and 70 (df= 1, 28, F= 6.923, p= 0.014) dBpeSPL respectively, by applying the ANOVA to test the significance of the regression model. It can be seen in

Figures 5.6 and **5.7** that as the amplitude of the SOAE increases the amplitude of the CEOAE increases.

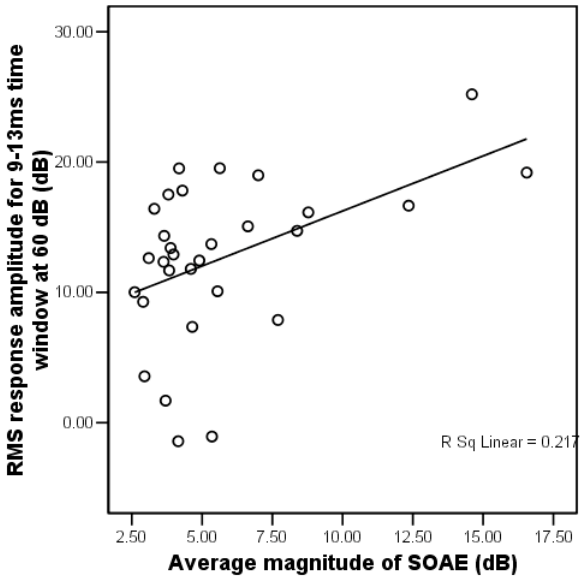


Figure 5.6

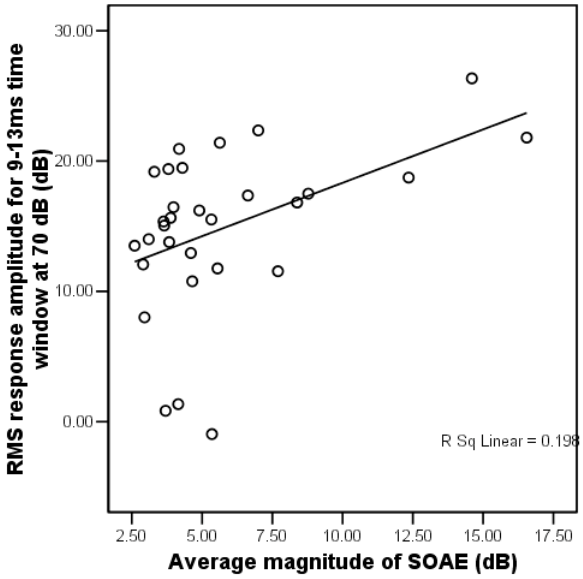
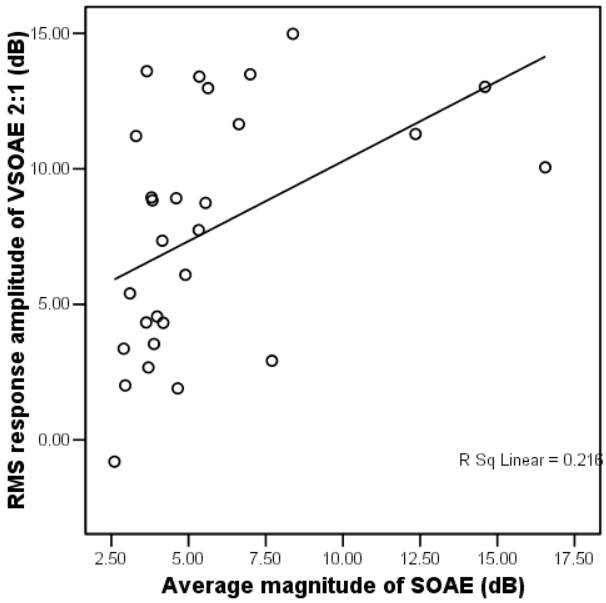


Figure 5.7

Figures 5.6 and 5.7. *The relationship between the amplitude of the CEOAE (conventional rate) for the 9-13ms time window at 60 dB (**Figure 5.6**) and 70 dB (**Figure 5.7**) respectively, with the average magnitude of the SOAE.*

To investigate whether the amplitude of the VSOAE second and third orders was related to the SOAE amplitude, the root mean square amplitude of the VSOAE S_{21} and S_{31}, at a stimulus rate of a 1000 clicks/s for the 2-6ms time window, was compared with the SOAE amplitude. **Figures 5.8** and **5.9** show that as the amplitude of the VSOAE increases the amplitude of the SOAE increases. This relationship was found to be significant on applying ANOVA to test the regression model, for both the S_{21}, and S_{31} slice waveforms (for S_{21} df= 1, 26, F= 7.156, p= 0.013; and for S_{31} df= 1, 26, F= 6.719, p= 0.015).



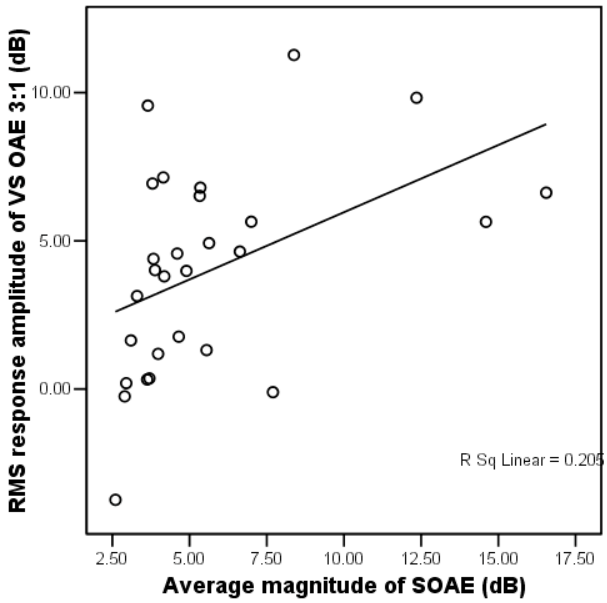


Figure 5.8 ***Figure 5.9***

Figures 5.8 and 5.9. *The interaction of the amplitude of S_{21} (**Figure 5.8**) and S_{31} (**Figure 5.9**), at a stimulus rate of a 1000 clicks/s (for the 2-6ms time window) with the amplitude of the SOAE.*

5.4.2) The relationship of VSOAE amplitude with CEOAE I/O function

Waveform correlations of greater than 0.5 were required for the CEOAE, conventional (VSOAE first order) responses in the 0-20ms time window. These responses were considered as being valid.

The amplitude nonlinearity was examined by calculating the slope of the I/0 function for all valid ear responses, at a conventional stimulus rate of 40 clicks/s. The I/O function was calculated for each ear by determining the interaction of the root mean square response amplitude of the CEOAE (conventional response) with the level tested between 50 and 70 dBpeSPL. A regression line was then fitted to all the points and the I/O slope function calculated for each ear. If the I/O function were linear when expressed in rms pressures then the slope, when expressed on dB-scales, would be 1 dB/dB. However, as the I/O function becomes more compressive the slope decreases therefore providing a measure of nonlinearity.[86]

Subsequently the measures of temporal nonlinearity, the amplitude of the S_{21} and S_{31} slice waveforms were examined, for the particular stimulus rate of a 1000 clicks/s and time window 2-6ms. For these VSOAEs correlations of >0.5 between repeat waveforms were an obligatory requirement for inclusion in the analysis.. The distribution for the VSOAE S_{21} and S_{31} slice waveforms was normal, as assessed by distribution curves and the Kolmogorov-Smirnov test, this can be seen in **Figures 5.10** and **5.11**.

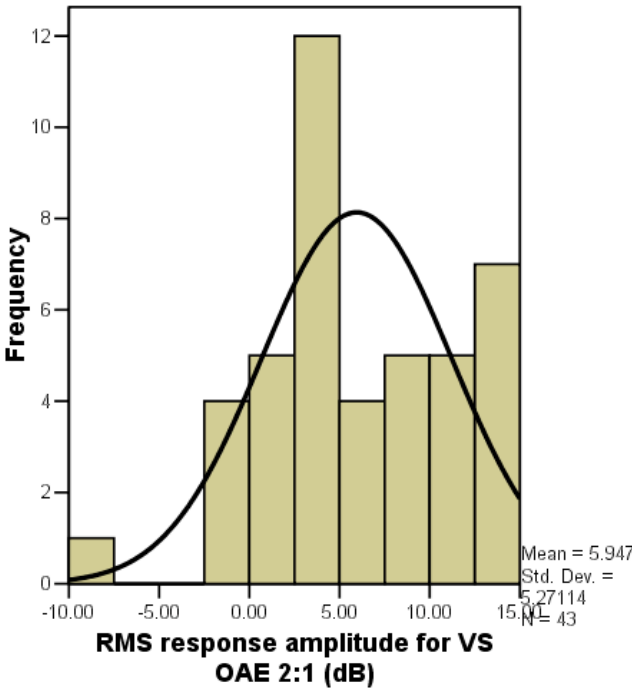


Figure 5.10

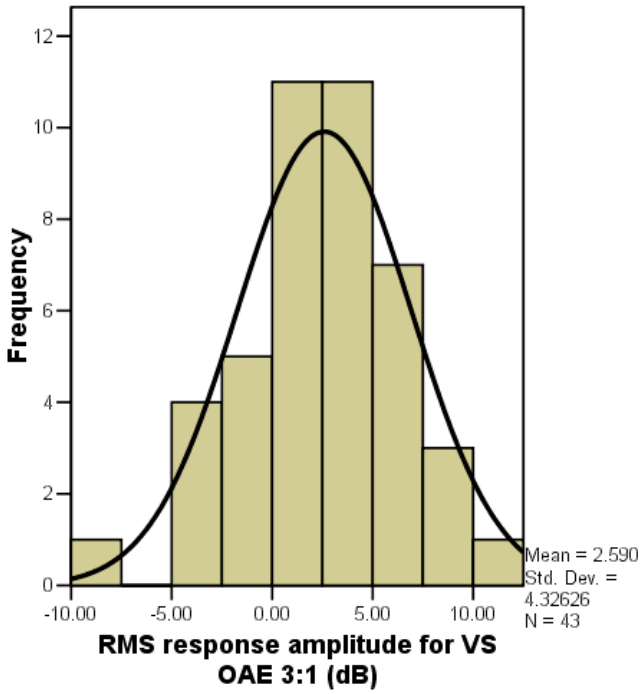


Figure 5.11

Figures 5.10 and 5.11. *Distribution for the VSOAE S_{21} (**Figure 5.10**) and S_{31} (**Figure 5.**11) slice waveforms respectively, at a stimulus rate of 1000 clicks/s for the 2-6ms time window.*

Hence the connection between the measure of amplitude nonlinearity and the measure of temporal nonlinearity was formulated. As such the slope of the I/O function for the CEOAE/CEOAE response was plotted against the S_{21} and S_{31} slice response amplitudes for each ear. There was a significant correlation between these two measures of nonlinearity for both the S_{21} (df= 1, 41, F= 7.682, p= 0.008) and S_{31} (df= 1, 41, F= 7.899, p= 0.008) slice waveforms in the 6-17ms time window as depicted in **Figures 5.12** and **5.13**. Thus as the amplitude of the VSOAE S_{21} and S_{31} increases, the slope of the I/O function decreases, representing an increase in the level of nonlinearity in the amplitude domain.

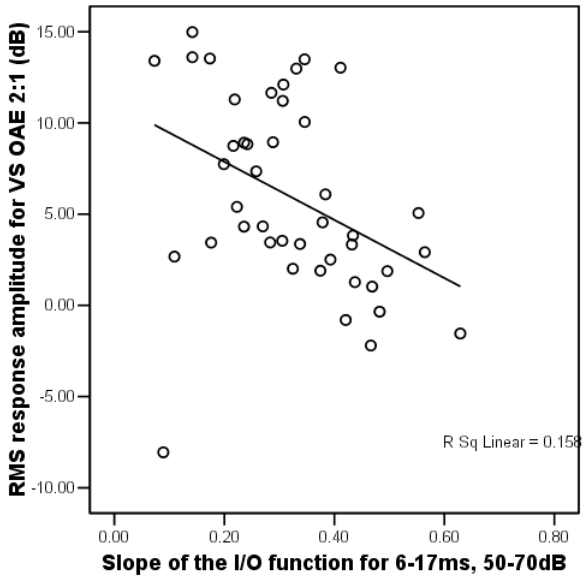


Figure 5.12

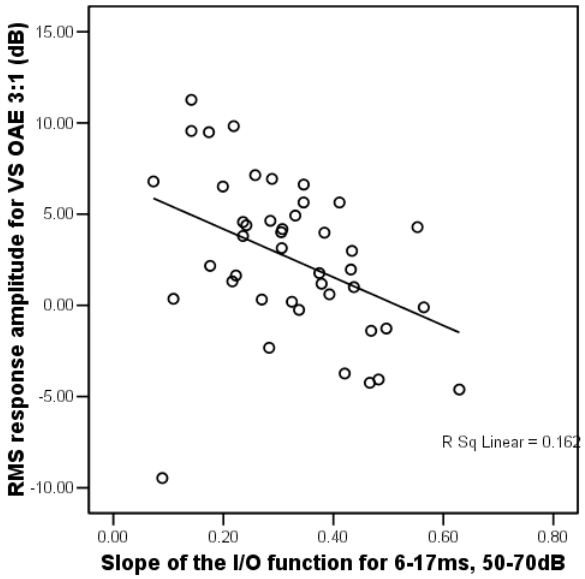


Figure 5.13

Figures 5.12 and 5.13. *The relationship between measures of amplitude and temporal nonlinearity, the RMS response amplitude for S_{21} (**Figure 5.12**) and S_{31} (**Figure 5.13**) respectively, with the slope of the I/O function for the 6-17ms time window averaged over 50-70 dBpeSPL.*

The association involving these two measures of nonlinearity was also tested for the 9-13ms time window. The same procedure as described above was carried out, however the values for the slope of the I/O function for the 9-13ms time window averaged between 50-70 dBpeSPL were substituted in the place of the values obtained for the 6-17ms time window. There was a significant effect between the RMS response amplitude of the VSOAE S_{21} (df= 1, 41, F= 5.505, p= 0.024) and S_{31} (df= 1, 41, F= 4.744, p= 0.035) slice waveforms respectively in the 9-13ms time window and the slope of the I/O function. This is demonstrated in **Figures 5.14** and **5.15**.

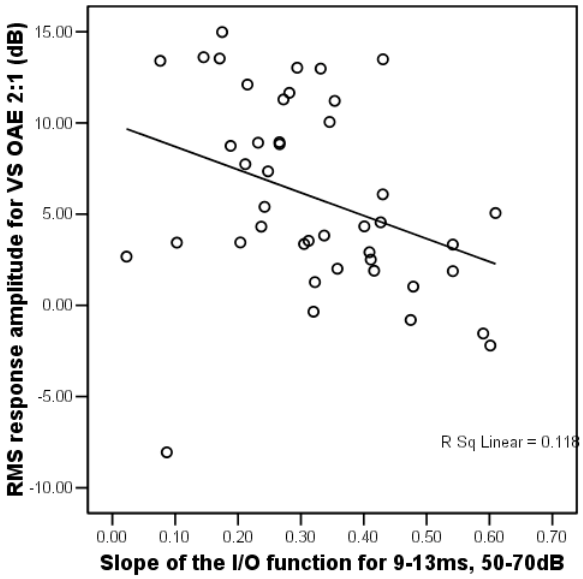


Figure 5.14

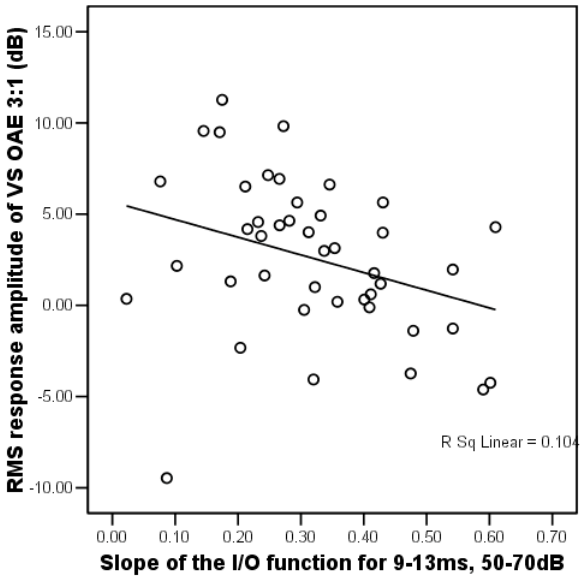


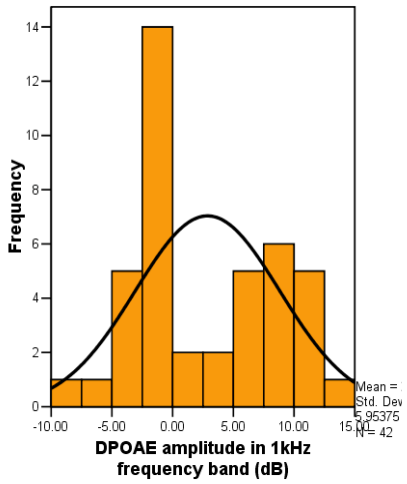
Figure 5.15

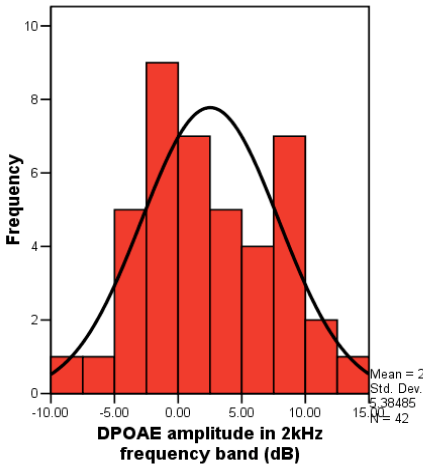
Figures 5.14 and 5.15. *The relationship between the measures of temporal and amplitude nonlinearity, the RMS response amplitude for S_{21} (**Figure 5.14**) and S_{31} (**Figure 5.15**) respectively, with the slope of the I/O function for the 9-13ms time window averaged over 50-70 dBpeSPL.*

Furthermore, in the 9-13ms time window as the degree of amplitude nonlinearity increases i.e. the slope of the I/O function decreases, the VSOAE S_{21} and S_{31} slice waveform amplitudes increase.

5.4.3) The relationship of I/O function with DPOAEs

In the third part of the experiment the link between the measure of nonlinearity in the frequency domain, i.e. DPOAEs and amplitude domain, i.e. the slope of the I/O function was studied. Two runs of the DPOAE measure were undertaken and the correlation between each run was calculated to ensure a viable response. The average DPOAE amplitude values calculated for individual ears were undertaken within three frequency octave bands of about 1kHz, 2kHz and 4kHz. Forty-two DPOAE responses were analysed in each bandwidth. The DPOAE response level was averaged only over responses with a SNR>6 dB; responses where the SNR was less than 6 dB were excluded, as this indicates a poor quality measurement. A high SNR indicates a good quality recording. The DPOAE recordings were normally distributed within each bandwidth, as shown below in **Figures 5.16**, **5.17** and **5.18**.





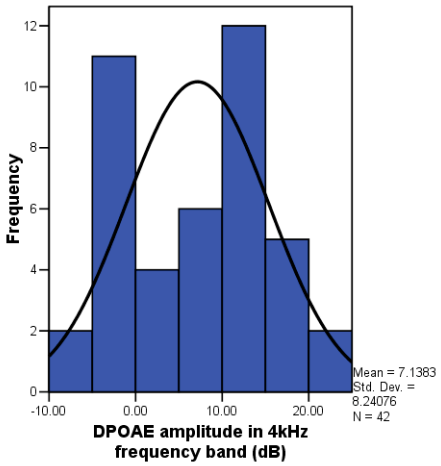


Figure 5.16 ***Figure 5.17*** ***Figure 5.18***

Figures 5.16, 5.17 and 5.18. *Distribution of responses for DPOAE amplitudes in bandwidths of 1kHz (**Figure 5.16**), 2kHz (**Figure 5.17**) and 4kHz (**Figure 5.18**) respectively.*

Valid DPOAE responses were obtained and analysed in 42 ears, in each of the frequency ranges. The interaction between the DPOAE with the slope of the I/O function was analysed for each bandwidth. No significant relationship was found

between the DPOAE and slope of the I/O function. **Table 5.0** shows the p values obtained.

Mean I/O Slope		DPOAE ~1kHz	DPOAE ~2kHz	DPOAE ~4kHz
6-17 ms	R Sig. (ANOVA)	0.13 0.42-	0.12 0.50	0.05 0.75
9-13 ms	R Sig. (ANOVA)	0.02 0.88	0.12 0.47	0.06 0.68

Table 5.0. *The significance of the interaction between the slope of the I/O function and DPOAE.*

5.4.4) The relationship of VSOAE second and third orders with DPOAEs

Ultimately, the relationship between the VSOAE best slices, S_{21} and S_{31}, waveform amplitudes, at a stimulus rate of 1000 clicks/s for the 2-6ms time window and the DPOAE amplitude was investigated. This was undertaken to see if there was a significant relationship between the measure of temporal nonlinearity (VSOAE 2nd and 3rd orders) and measure of nonlinearity in the frequency domain (DPOAE). This was performed for each of the near octaves. These results have been plotted in **Figures 5.19** to **5.21** for the VSOAE S_{21} and in **Figures 5.22** to **5.24** for the S_{31}.

There appears to be no significant relationship between the S_{21} and S_{31}, slice amplitudes and the DPOAE amplitude in any of the frequency bands tested, these results have been summarised in **Table 5.1**.

RMS response amplitude of VSOAE		DPOAE ~1kHz	DPOAE ~2kHz	DPOAE ~4kHz
S_{21}	R Sig. (ANOVA)	-0.17 0.28	-0.04 0.81	-0.04 0.82
S_{31}	R Sig. (ANOVA)	-0.17 0.29	0.07 0.69	0.04 0.79

***Table 5.1**. The significance of the interaction between the RMS response amplitude of VSOAE S_{21} and S_{31} and DPOAEs in the different frequency bands.*

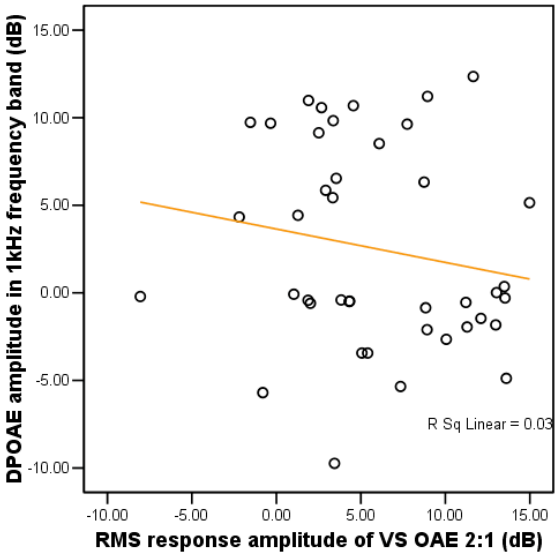


Figure 5.19

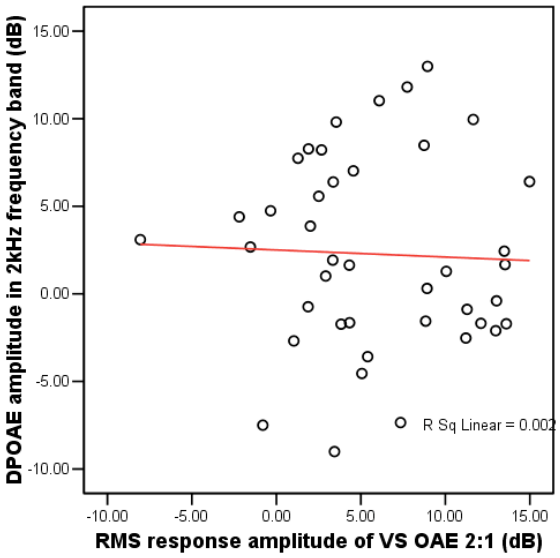


Figure 5.20

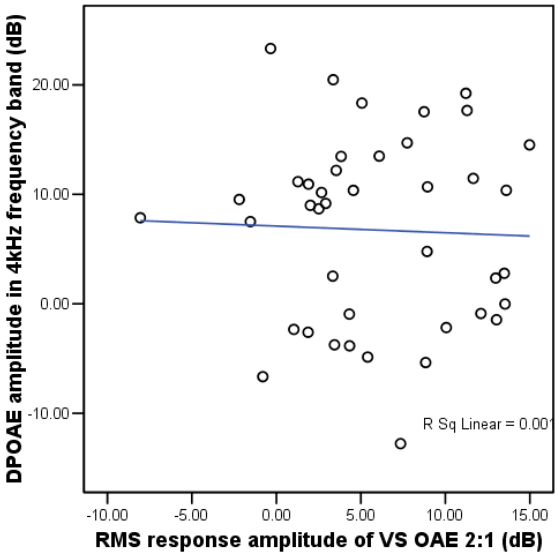
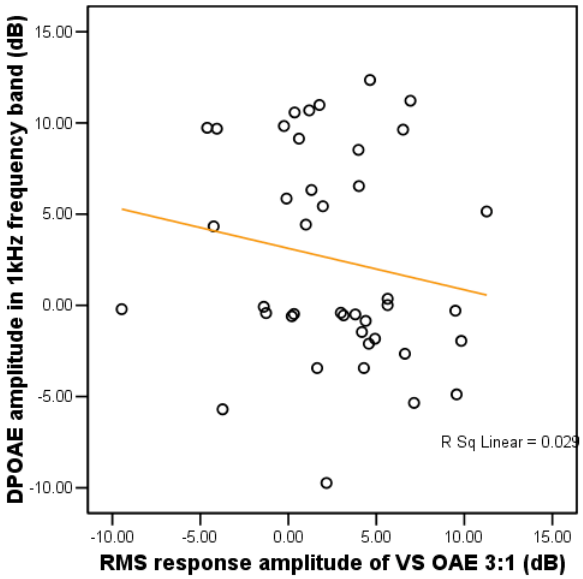


Figure 5.21

***Figures 5.19, 5.20 and 5.21**. The relationship between the DPOAE amplitude for the ~1kHz (**Figure 5.19**), ~2kHz (**Figure 5.20**) and ~4kHz (**Figure 5.21**) frequency bands respectively and the RMS response amplitude for the VSOAE S_{21} slice waveform.*



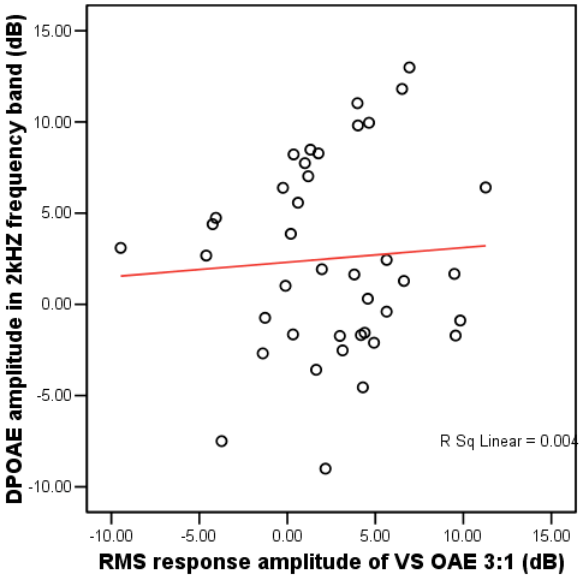


Figure 5.22

Figure 5.23

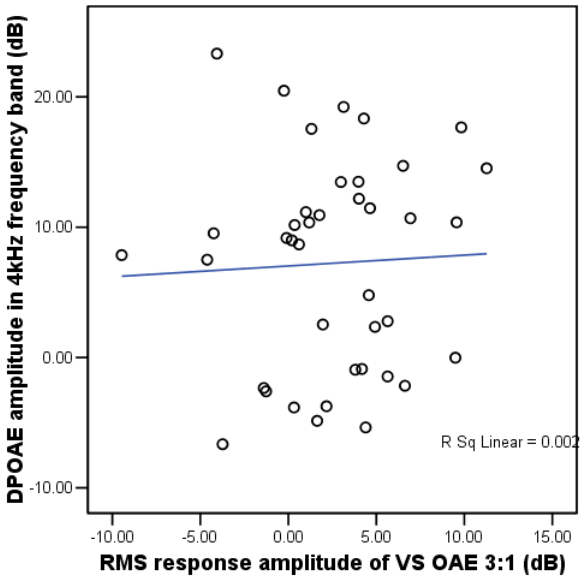


Figure 5.24

Figures 5.22, 5.23 and 5.24*. The relationship between the DPOAE amplitude for the ~1kHz (**Figure 5.22**), ~2kHz (**Figure 5.23**) and ~4kHz (**Figure 5.24**) frequency bands and the RMS response amplitude for the VSOAE S_{31} slice waveform.*

The data was then split into two groups. Those responses in SOAE positive ears, and those responses in SOAE negative ears. The results for all the slices of the VSOAE S_2 and S_3, at all stimulus rates, with enough valid responses were then reanalysed. Pearson's correlation coefficient was used to test the significance of the relationship between the two variables.

When SOAEs were absent, the root mean square amplitude of the VSOAE S_{32} (3^{rd} order 2^{nd} slice), for the 2-6 ms time window, recorded at a stimulus rate of 1200 clicks/s, where 10 valid responses were analysed, was significantly related to the DPOAE response in the 1 kHz near bandwidth (p= 0.050). This relationship is demonstrated in **Figure 5.25**. An inverse relationship between the DPOAE and VSOAE is shown; as the DPOAE amplitude increases, the VSOAE S_{32} amplitude decreases.

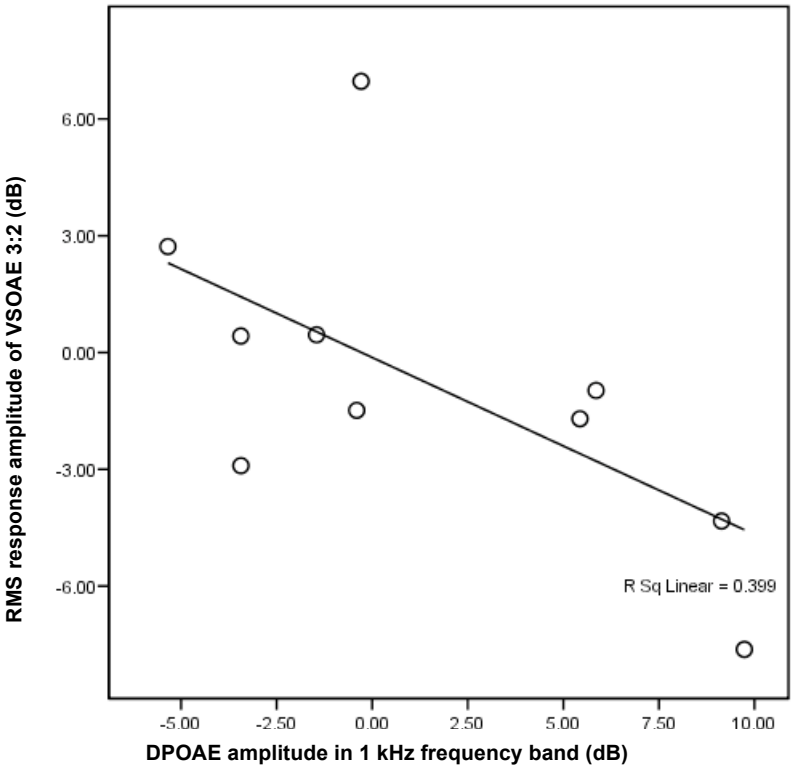


Figure 5.25. *The relationship between the RMS response amplitude for the VSOAE S_{32} slice waveform, obtained at a stimulus rate of 1200 clicks/s, in SOAE-negative ears and the DPOAE amplitude for the ~1kHz frequency band.*

For subjects in which SOAEs were recorded, there were significant relationships between the VSOAE S_{22} and VSOAE $S_{23,}$ obtained at a stimulus rate of 1000 clicks/s respectively and the DPOAE response. For the third order VSOAE there was a significant relationship between the VSOAE S_{32} and the DPOAE response for stimulus rates of 800 and 1200 clicks/s. For the VSOAE $S_{22,}$ 18 valid responses were included, and the relationship was significant with the DPOAE response at the near octaves of 1 kHz (p= 0.006) and approaching significance at 2 kHz (p= 0.063). The results for the VSOAE S_{22} and the DPOAEs in the 1 and 2 kHz bandwidths are shown in **Figures 5.26** and **5.27**.

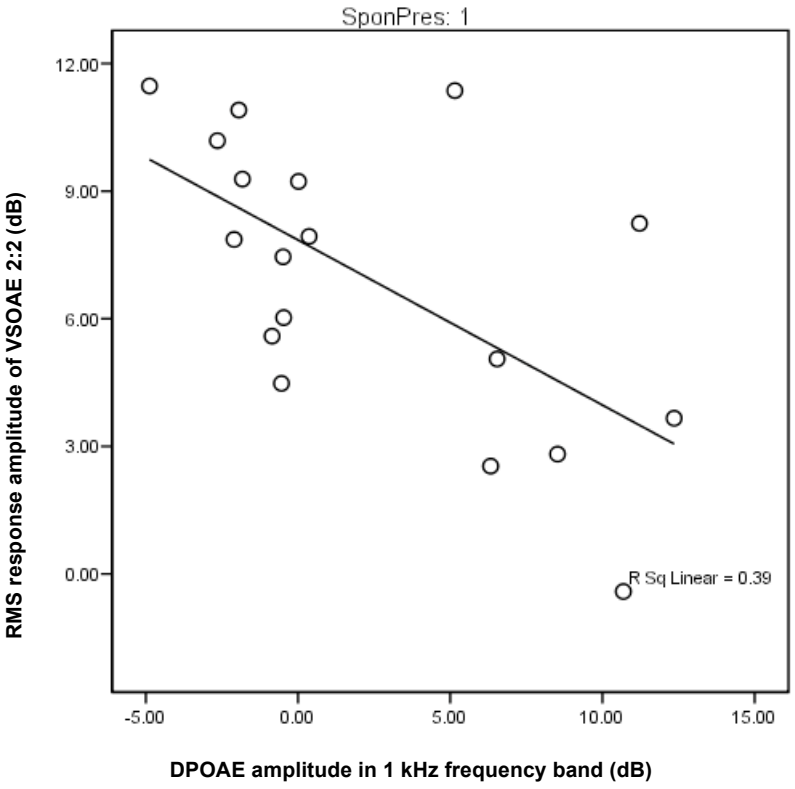


Figure 5.26. *The relationship between the RMS response amplitude for the VSOAE S_{22} slice waveform, obtained at a stimulus rate of 1000 clicks/s, in SOAE-positive ears and the DPOAE amplitude for the ~1kHz frequency band.*

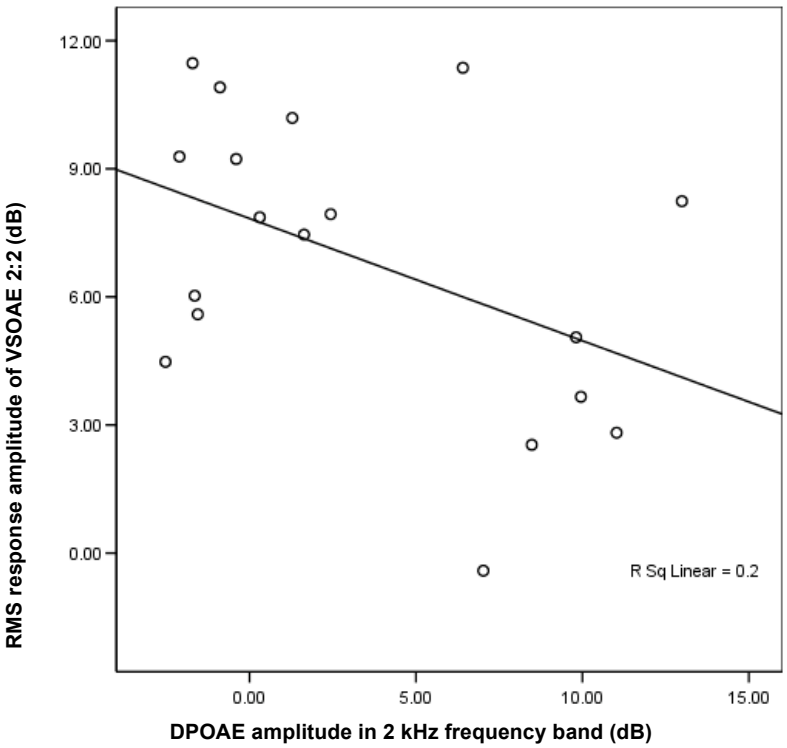


Figure 5.27. *The relationship between the RMS response amplitude for the VSOAE S_{22} slice waveform, obtained at a stimulus rate of 1000 clicks/s, in SOAE-positive ears and the DPOAE amplitude for the ~1kHz frequency band.*

Both these figures show that as the DPOAE amplitude increases the amplitude of the VSOAE decreases. In the case of the VSOAE S_{23}, where 15 responses were analysed, there was a significant interaction with the DPOAE response in the 1 kHz ($p= 0.004$) and 2 kHz ($p= 0.015$) near octaves. The results for the VSOAE S_{23} are shown in **Figures 5.28** and **5.29** and as can be seen as the DPOAE amplitude increases the VSOAE amplitude decreases.

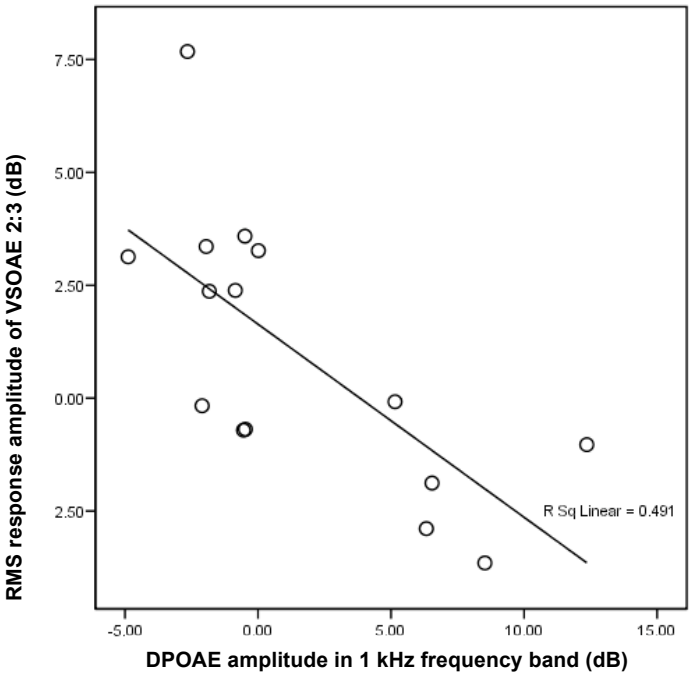


Figure 5.28.

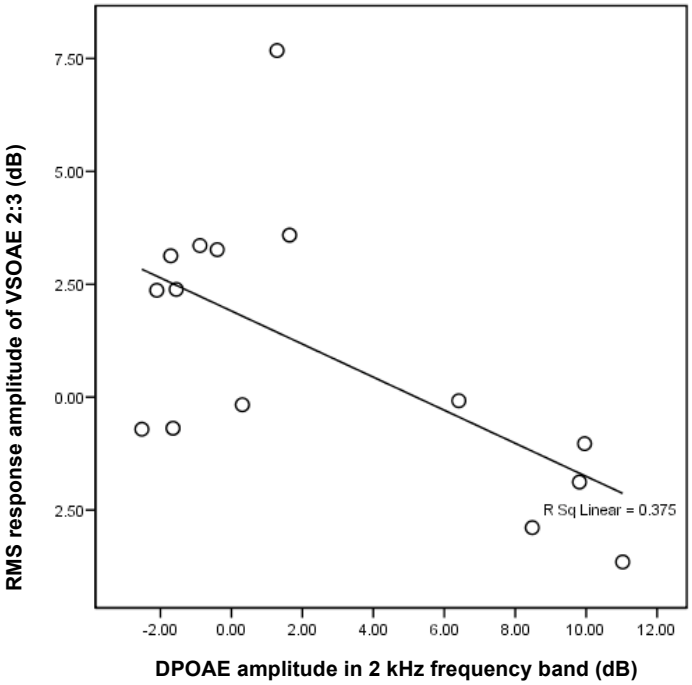


Figure 5.29.

Figures 5.28 & 5.29. *The relationship between the RMS response amplitude for the VSOAE S_{23} slice waveform, obtained at a stimulus rate of 1000 clicks/s, in SOAE-positive ears and the DPOAE amplitudes for the ~1 kHz (**figure 5.28**) and ~2kHz (**figure 5.29**) frequency bands.*

The VSOAE S_{32} obtained at a stimulus rate of 800 clicks/s was significantly correlated with the DPOAE responses in the 1 kHz (p= 0.030) and 2 kHz (p= 0.046) near octaves. Twelve responses were included in the analysis of the VSOAE S_{32} (obtained at a stimulus rate of 800 clicks/s). The results for the VSOAE S_{32} are depicted in **Figures 5.30** to **5.33**. Finally the VSOAE S_{32} obtained at a stimulus rate of 1200 clicks/s where SOAEs were present and 16 responses were analysed was shown to be significantly related to the DPOAE in the 1 kHz (p= 0.012) and 2 kHz (p= 0.029) near octaves. Furthermore, in these cases an inverse relationship between the VSOAE and DPOAE amplitudes was seen.

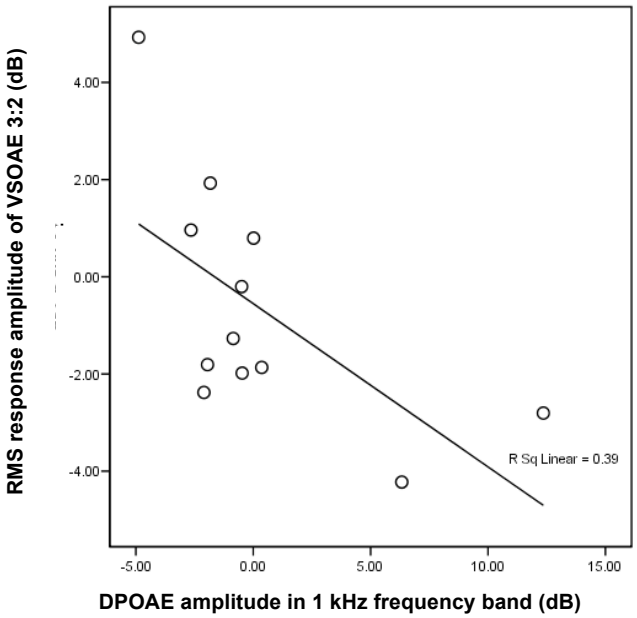


Figure 5.30

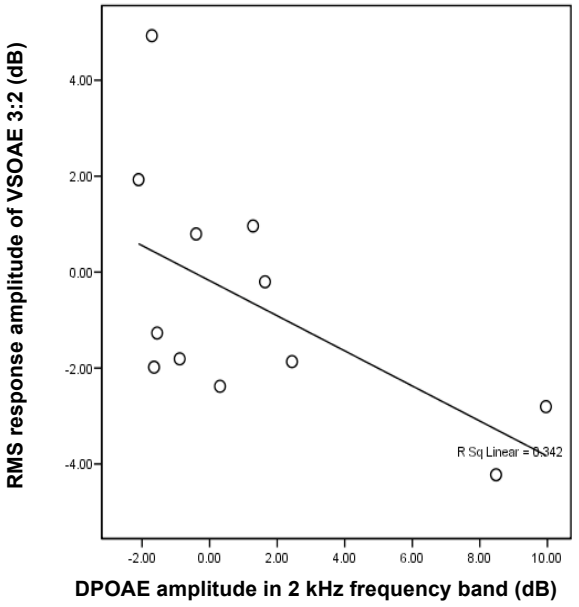


Figure 5.31

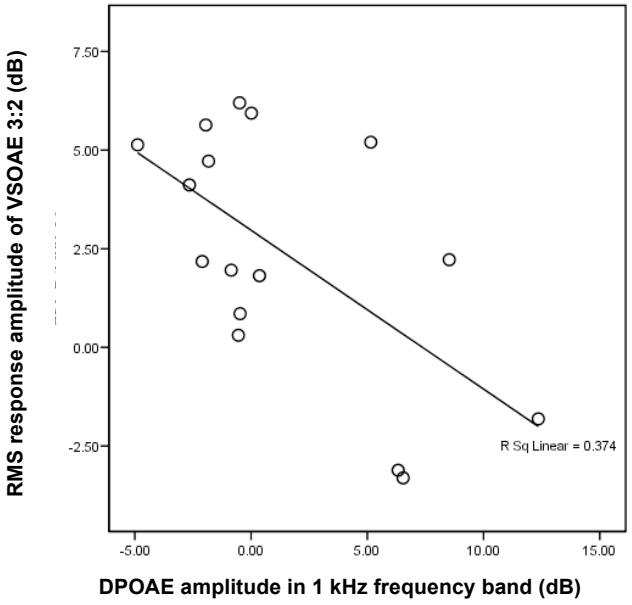


Figure 5.32

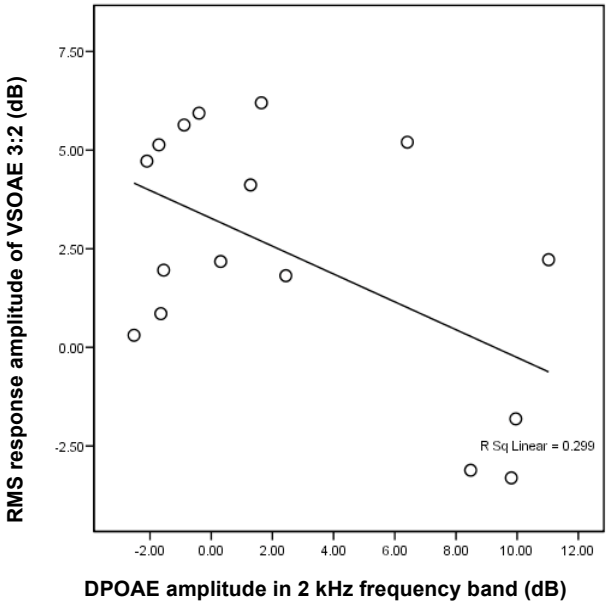


Figure 5.33

Figures 5.30 to 5.33. *The relationship between the RMS response amplitude for the VSOAE S_{32} slice waveforms, obtained at a stimulus rates of 800 clicks/s (**figures 5.30 & 5.31**) and 1200 clicks/s (**figures 5.32 & 5.33**), in SOAE-positive ears and the DPOAE amplitude for the ~1 kHz (**figures 5.30 & 5.32**) and ~2 kHz (**figures 5.31 & 5.33**) frequency bands respectively.*

5.4.4) The interaction of VSOAEs, SOAEs, CEOAE I/O functions and DPOAEs

As the VSOAE, SOAE, CEOAE I/O function and the DPOAE are all thought to arise from nonlinear processes within the cochlea, the relationship of the VSOAE with these measures of nonlinearity was examined. Using correlation techniques the significance of this link was tested for the S_{21} and S_{31} slice waveforms at a stimulus rate of 1000 clicks/s, for the 2-6ms time window. The independent variables tested were varied with the relationship being tested with the slope of the I/O function between 50 and 70 dBpeSPL, in both the 6-17 and 9-13ms time windows, and for each DPOAE frequency band tested. The results have been summarised in **Tables 5.2 and 5.3**. The results indicate that there is a significant correlation between both the VSOAE S_{21} and VSOAE S_{31} slice waveforms and the slope of the I/O function in both time windows. There is a significant correlation between the VSOAE S_{31} and DPOAE in the 1 kHz near band. There are also correlations which approach significance between the VSOAE S_{21} and VSOAE S_{31} and the SOAE, and VSOAE S_{21} and the DPOAE in the 1 kHz near band.

		VSOAE S_{21}	SOAE amplitude	Slope I/O function (6-17 ms, 50-70 dBpeSPL)	Slope I/O function (9-13 ms, 50-70 dBpeSPL)	DPOAE		
						1kHz	2kHz	4kHz
VSOAE S_{21}	Pearson Correlation	1	.414	-.558(**)	-.438(**)	-.299	-.093	-.085
	Sig. (2-tailed)	40	**.088**	**.000**	**.005**	**.068**	.579	.611
	N		18	40	40	38	38	38
SOAE amplitude	Pearson Correlation	.414	1	.244	.005	-.251	-.178	-.176
	Sig. (2-tailed)	**.088**	18	.329	.983	.315	.480	.484
	N	18		18	18	18	18	18
Slope I/O function (6-17 ms, 50-70 dBpeSPL)	Pearson Correlation	-.558(**)	.244	1	.873(**)	.208	-.045	.121
	Sig. (2-tailed)	**.000**	.329	40	**.000**	.211	.788	.470
	N	40	18		40	38	38	38
Slope I/O function (9-13 ms, 50-70 dBpeSPL)	Pearson Correlation	-.438(**)	.005	.873(**)	1	.142	-.055	.046
	Sig. (2-tailed)	**.005**	.983	**.000**	40	.396	.741	.785
	N	40	18	40		38	38	38
DPOAE 1kHz	Pearson Correlation	-.299	-.251	.208	.142	1	.878(**)	.554(**)
	Sig. (2-tailed)	**.068**	.315	.211	.396	38	**.000**	**.000**
	N	38	18	38	38		38	38
DPOAE 2kHz	Pearson Correlation	-.093	-.178	-.045	-.055	.878(**)	1	.550(**)
	Sig. (2-tailed)	.579	.480	.788	.741	**.000**	38	**.000**
	N	38	18	38	38	38		38
DPOAE 4kHz	Pearson Correlation	-.085	-.176	.121	.046	.554(**)	.550(**)	1
	Sig. (2-tailed)	.611	.484	.470	.785	**.000**	**.000**	38
	N	38	18	38	38	38	38	

** Correlation is significant at the 0.01 level (2-tailed).

Table 5.2. *The correlations of the amplitude of the S_{21} slice waveform with the SOAE amplitude, slope of the I/O function (for different time windows) and the DPOAE amplitude (for different frequency bands).Bold print and underline indicates significant result or result approaching significance.*

		VSOAE S_{31}	SOAE amplitude	Slope I/O function (6-17 ms, 50-70 dBpeSPL)	Slope I/O function (9-13 ms, 50-70 dBpeSPL)	DPOAE		
						1kHz	2kHz	4kHz
VSOAE S_{31}	Pearson Correlation	1	.467	-.614(**)	-.473(**)	-.339	-.067	-.056
	Sig. (2-tailed)	34	**.079**	**.000**	**.005**	**.054**	.712	.757
	N		15	34	34	33	33	33
SOAE amplitude	Pearson Correlation	.467	1	.230	-.061	-.005	-.051	.046
	Sig. (2-tailed)	**.079**	15	.410	.829	.985	.858	.870
	N	15		15	15	15	15	15
Slope I/O function (6-17 ms, 50-70 dBpeSPL)	Pearson Correlation	-.614(**)	.230	1	.887(**)	.208	-.071	.132
	Sig. (2-tailed)	**.000**	.410	34	**.000**	.245	.695	.465
	N	34	15		34	33	33	33
Slope I/O function (9-13 ms, 50-70 dBpeSPL)	Pearson Correlation	-.473(**)	-.061	.887(**)	1	.124	-.060	.077
	Sig. (2-tailed)	**.005**	.829	**.000**	34	.493	.741	.669
	N	34	15	34		33	33	33
DPOAE 1kHz	Pearson Correlation	-.339	-.005	.208	.124	1	.886(**)	.555(**)
	Sig. (2-tailed)	**.054**	.985	.245	.493	33	**.000**	**.001**
	N	33	15	33	33		33	33
DPOAE 2kHz	Pearson Correlation	-.067	-.051	-.071	-.060	.886(**)	1	.559(**)
	Sig. (2-tailed)	.712	.858	.695	.741	**.000**	33	**.001**
	N	33	15	33	33	33		33
DPOAE 4kHz	Pearson Correlation	-.056	.046	.132	.077	.555(**)	.559(**)	1
	Sig. (2-tailed)	.757	.870	.465	.669	**.001**	**.001**	33
	N	33	15	33	33	33	33	

** Correlation is significant at the 0.01 level (2-tailed).

Table 5.3. *The correlations of the amplitude of the S_{31} slice waveform with the SOAE amplitude, slope of the I/O function (for different time windows) and the DPOAE amplitude (for different frequency bands).Bold print and underline indicates significant result or result approaching significance.*

5.5) Discussion of the relationship of VSOAEs to existing nonlinear OAE measures

5.5.1) The relationship of VSOAE amplitude with SOAE amplitude

A significant relationship was demonstrated between the amplitudes of both the CEOAE (linear response, for the 6-17 ms and 9-13 ms time windows) and also the VSOAEs S_{21} and S_{31} and the SOAE amplitude. As previously discussed in **Chapter 4**, ears in which SOAEs are found have VSOAEs of greater amplitude than those where SOAEs cannot be recorded. However, here we clearly see that there a significant correlation between the amplitudes of the responses, such that as the SOAE amplitude increases the VSOAE amplitude increases.

The presence of the SOAE depends on the identification of a spectral peak and SOAEs were considered valid if the amplitude exceeded the noise floor by 3 dB or more as has been used in prior research.[154] Four recordings for each sweep for the SOAE were also repeated for each ear, in order to assess repeatability and assess the stability of the responses. In this current study 400 sweep spectra were averaged for detection of SOAEs to improve the results; this in comparison to other studies where 10 and 100 sweep spectra have been averaged.[130, 154]

SOAEs are thought to be generated at specific sites along the cochlear partition and that a structurally unique feature of the organ of Corti is most likely involved in their generation.[14] Three different mechanisms for their origin have been suggested.[155] Based on the work of Thomas Gold, the local-oscillator model supposes that SOAEs result from the local, autonomous oscillation of some cellular constituent of the organ of Corti (e.g. the ‘active process underlying the cochlear amplifier).[155] Both of the alternative models suggest that SOAEs are ‘a global collective phenomenon- cochlear standing waves created by multiple internal reflection’, but these models differ with regards to the likely source.[155] The ‘passive’ standing-wave model supposes that SOAEs are biological noise, passively amplified

by cochlear standing-wave resonances acting as narrow band nonlinear filters.[155] The ‘active’ standing-wave model proposes that standing-wave amplitudes are actively maintained by coherent wave amplification within the cochlea.[155] The amplitudes of SOAEs do not generally exceed 20 dB SPL; an explanation for this is that there exists an innate self limiting saturation mechanism that restricts the generation of high level emissions.[14] SOAE interaction phenomena have also been demonstrated in ears with several SOAEs, and have been shown to be generated as distortion products of two other SOAEs; these interactions most likely being related to the inherent nonlinear behaviour of SOAEs.[14] The underlying mechanism for these reactions is poorly understood.[14] However, Shera has shown that SOAEs are likely to be ‘amplitude-stabilised standing waves produced by the cochlea acting as a biological, hydromechanical analogue of a laser oscillator’, as accounted for by the active standing-wave model.[155]

Thus, if SOAEs originate from nonlinear processes within the cochlea, it is likely that they would correlate significantly with the nonlinear VSOAEs, and this was shown to be the case. However, a significant relationship between the amplitude of the CEOAE (linear response) and the amplitude of the SOAE was also found. This may be explained by the finding that ears with SOAEs have been shown to have CEOAE emissions of greater amplitude, and also a greater number of valid responses, than those without SOAEs, as was observed in **Chapter 4**. The effect of SOAEs on transiently evoked otoacoustic emissions (TEOAEs) has previously been studied. Significantly greater TEOAEs have been exhibited in ears with measurable SOAEs, supporting the finding that the amplitude of the CEOAE is increased in SOAE-positive ears.[156] Healthy newborns have also been found to have higher amplitude evoked OAEs in ears with SOAEs, providing further evidence that these increased amplitude emissions are partly due to the increased incidence of SOAEs, (78%) observed in this age group.[154, 157] In their study Kulawiec et al, 1995 found that overall the TEOAE response level was also greater in ears with SOAEs.[158] Thus these results support the current finding of an increased CEOAE amplitude in the presence of SOAEs, and

also may imply that there is a positive correlation between the amplitude of the CEOAE and SOAE.

The presence of SOAEs has been linked both with cochlear health and also pathology.[14] However, hearing loss at higher frequencies (16 kHz) has been shown to be significantly smaller in ears with SOAEs than in ears without SOAEs, supporting the theory that they are an indicator of cochlear health.[159] Thus, those with SOAEs are more likely to have more detectable otoacoustic emissions of other types. This is supported by the fact that females generally exhibit more SOAEs than males, a sex difference present from birth; and also right ears more SOAEs than left ears and TEOAE (and CEOAE) amplitudes have been shown to be greater in females and in right ears (see **Chapter 3**). Indeed larger DPOAEs have been recorded from ears exhibiting SOAEs.[154] Thus these emissions may originate from a common cochlear focus.

Volterra slices are very reliable and sensitive measures of the degree of nonlinearity of the cochlea.[100] As described SOAEs may also originate from nonlinear processes, therefore a positive correlation between the amplitudes of these two measures is likely, and provides an explanation as to why an increased SOAE amplitude is significantly correlated with increased VSOAE amplitude. The factors mentioned in the previous paragraph must also be considered. The relationship between the SOAE and VSOAEs S_{21} and S_{31} was similar, with the exception of a greater amplitude obtained for the VSOAE S_{21} waveform as is consistent with previous findings by this group, and in **Chapter 4**.[80]

Thus the reasons why increasing SOAE amplitude may be correlated both with an increased CEOAE and VSOAE amplitude have been described.

5.5.2) The relationship of VSOAE amplitude with CEOAE I/O function

There was a significant correlation between the RMS response amplitude of the VSOAE S_{21} and S_{31} slice waveforms respectively (in the 6-17 ms and 9-13 ms time windows) and the slope of the I/O function. Thornton et al (2006) showed that in a cohort of 12 subjects, in which 18 ears were tested that the degree of amplitude nonlinearity was related to the amplitude of the volterra slices. The degree of nonlinearity increased with the level of the VSOAE S_{21} and S_{31} components.[86] This result tallies with the findings of this current study in a larger cohort. However, in this current study we included those subjects with SOAEs, although the spectral peak was not always greater than 5 dB and still found this significant correlation to be the case. It has been suggested that SOAEs can modify the TEOAE response, and therefore the I/O function of CEOAEs.[160] Tavartkiladze et al (1994) demonstrated that in the one subject with a recordable SOAE in their study that the maximal click evoked OAE duration was observed. They also showed a clear relationship between the latency and input/output curve shape for all subjects (including the subject with an SOAE); the longer the latency of the TEOAE components the I/O function became more nonlinear and saturated.[149] In addition it was shown that significant suppression of TEOAE generation with a latency of 8 ms or longer was present in the one subject out of five with the SOAE, which may support the theory that the presence of strong SOAEs alter I/O function.[149] However, this may also be a spurious result from one subject, and also the presence of SOAEs in the general population is higher, so other subjects may have had weaker SOAEs not detected although a sound experimental technique appears to have been used with the ILO88 and ILO92 (Otodynamics Ltd).[149]

A model for nonlinear temporal interactions in CEOAEs was proposed by Kapadia and Lutman (2001). ‘CEOAEs are reduced in amplitude by suppressor clicks that either closely lead or follow the stimulus (test) clicks’, this suppression of the response represents nonlinear temporal interactions between test and suppressor clicks.[161] Their model was composed of a static nonlinearity, representing the outer

hair cell nonlinearity, preceded by a band pass filter, representing the ringing of a region of the basilar membrane.[86, 162] The rate suppression has been found to be significantly related to the amplitude nonlinearity as measured by the slope of the I/O function, as predicted by the model.[100] Thus the use of the slope of the I/O function as the measure for amplitude nonlinearity in this current study.

Thus there is a relationship between the nonlinearity of the cochlea and the magnitude of the Volterra slices obtained from it. There is an increase in amplitude of the S_{21} and S_{31}, as the linearity of the system decreases (or degree of nonlinearity increases) as shown by the negative gradients (**Figures 5.12 to 5.15**). It was likely that as the I/O function represents nonlinearity in the amplitude domain, and that higher order VSOAEs (i.e. not S_{11}) represent nonlinearity in the temporal domain that the two would be related, and the results support this. These results also add weight to the hypothesis that these different nonlinear phenomena arise from a common origin.

5.5.3) The relationship of CEOAE I/O function with DPOAEs

There was no significant relationship demonstrated between the slope of the I/O function and the DPOAEs.

The DPOAE represents a measure of nonlinearity of the system in the frequency domain and the I/O function amplitude nonlinearity, hence a significant correlation between these two measures would be expected. By definition DPOAEs represent evoked nonlinear responses as they consist of new frequencies absent in the eliciting stimulus.[14] In this study valid DPOAEs were obtained from 42/45 (93%) ears. There is evidence that DPOAEs are a property of all normally hearing human ears, with findings that they can be recorded in over 90% of normal ears, consistent with the current findings.[22, 110, 163] They are detected in the same frequency range as other classes of OAEs between 1-8 kHz.[14] The DPOAE amplitude depends on the frequencies of the primaries, separation ratio and innate properties of each ear.[14,

22] The most effective f2/f1 ratio for eliciting DPOAEs from 1 to 4 kHz has been reported as 1.22.[154, 164] In these experiments the frequencies and ratios were selected so as to obtain the most valid responses, and corresponded to these ideal values, although the ratio used by Moulin et al (1993) was 1.17.[130, 154] DPOAE amplitudes are also influenced by SOAEs, TEOAEs and SFOAEs.[14] Larger DPOAEs have been obtained from ears exhibiting SOAEs, and in this current study the majority of ears had SOAEs.[154] This finding would suggest that the group of subjects in the current study had larger DPOAEs, but this does not explain why no relationship with the measure of amplitude nonlinearity was found. Although 'distortion product SOAEs'; DPOAEs evoked by two coexisting SOAEs, have been described, it is unlikely these played a role as the amplitudes of the SOAEs recorded in this current study were not particularly large to result in this effect.[14] TEOAEs appear to closely correspond with DPOAEs, and there appears to be a high correspondence between the distribution of the energy for each emission and audiometric threshold levels at corresponding frequencies, suggesting that both TEOAEs and DPOAEs are derived largely from similar mechanisms.[165] Experimental findings have suggested that the detailed mechanism of TEOAE and all DPOAEs is very similar when close stimulus tones are used to stimulate DPOAEs.[166] Thus, it is widely assumed that OAEs of all types arise by a common mechanism: nonlinear electromechanical distortion within the cochlea.[81] However, Shera & Guinan (1999) suggest that evoked emissions arise by two fundamentally different generation mechanisms in the cochlea these being; linear reflection versus nonlinear distortion.[81] As such, two broad classes of emissions 'reflection-source' and 'distortion-source' emissions can be distinguished based on the mechanisms of their generation.[81] Significant divergence exists with the $2f_1 - f_2$ DPOAE with the wider stimulus ratios typically employed for clinical testing.[166] Thus there may have been no correspondence between the two measures due to the selected parameters chosen to optimize the DPOAE output level. DPOAE amplitudes are known to be smaller in ears with high frequency hearing losses; however again this is unlikely to have affected the result as DPOAE amplitudes obtained were good and subjects

were tested under experimental conditions and found to be of normal hearing up to 8 kHz.

As mentioned in **section 5.5.2** strong SOAEs may alter the I/O function of CEOAEs.[100, 149] Indeed in our current study 9 subjects had spectral peaks greater than 5 dB. However, as SOAEs were present in the majority of valid responses, excluding subjects with SOAEs would have resulted in too small a sample size. Therefore, this phenomenon may have caused this unexpected result; however, in the study quoted this effect was only in one subject. Furthermore, in another study of CEOAE I/O functions, obtained from 223 ears of normally hearing adult subjects differences were independent of level and SOAE status, but were dependent on frequency.[167]

These data indicate significant correlations between temporal and amplitude nonlinearities and no significant correlations for frequency domain nonlinearities (SOAEs and DPOAEs). The implication being that the amplitude and temporal nonlinearities arise from the same generators, whereas the frequency domain nonlinearities have different generators.

5.5.4) The relationship of VSOAE second and third orders with DPOAEs

There was no significant relationship between the VSOAE S_{21} and S_{31} amplitudes and the DPOAE amplitude in any of the frequency bands tested.

However, in the absence of SOAEs the VSOAE S_{32} amplitude (recorded at a stimulus rate of 1200 clicks/s), was significantly related to the DPOAE response in the 1 kHz near bandwidth. In this analysis there where only 10 valid responses. The relationship showed that as the DPOAE amplitude increased, the VSOAE S_{32} amplitude decreased. This is the converse of what would be expected. It would be reasonable to assume that the measure of nonlinearity in the temporal domain would be related to the measure of nonlinearity in the frequency domain, thus an increase in the DPOAE

amplitude would result in an increase in the VSOAE amplitude. This of course may not be the case; however, caution must be applied to this result due to the small sample size.

When SOAEs were present, there were significant relationships between the VSOAE S_{22} and VSOAE S_{23} (obtained at a stimulus rate of 1000 clicks/s respectively) and the DPOAE response. There was also a significant relationship between the VSOAE S_{32} and the DPOAE response for stimulus rates of 800 and 1200 clicks/s. For the VSOAE S_{22}, the relationship was significant with the DPOAE response at the near octaves of 1 kHz and 2 kHz. A sufficient number of valid responses (18) were included. As the DPOAE amplitude increased the amplitude of the VSOAE decreased. In the case of the VSOAE S_{23}, where 15 responses were analysed, there was a significant interaction with the DPOAE response in the 1 kHz, 2 kHz and 4 kHz near octaves. Once again as the DPOAE amplitude increased the VSOAE amplitude decreased. The VSOAE S_{32} obtained at a stimulus rate of 800 clicks/s (12 responses) was significantly correlated with the DPOAE responses in the 1 kHz and 2 kHz near octaves. Finally, the VSOAE S_{32} obtained at a stimulus rate of 1200 clicks/s where SOAEs were present and 16 responses were analysed was shown to be significantly related to he DPOAE in the 1 kHz and 2 kHz frequency bands. Furthermore, in these cases an inverse relationship between the VSOAE and DPOAE amplitudes was seen.

In order to attempt to explain these results several factors must be taken into account. The earlier results (**section 5.4.1**) showed that as the amplitude of the SOAE increased, the amplitude of the VSOAE increased. Thus the presence of the SOAE was positively correlated with an increased VSOAE, and their presence reflects an increased nonlinearity of the cochlea. In addition, larger DPOAEs have been demonstrated in ears exhibiting SOAEs, than in ears without measurable SOAEs, suggesting that SOAEs play an additive role in the measurement of DPOAEs.[154] Ozturan et al (1999) measured the mean DPOAE amplitude in response to each primary-tone level (40 dB SPL- 70 dB SPL) in SOAE-positive ears and compared this to the corresponding mean DPOAE amplitude level in SOAE-negative ears (obtained

at the same corresponding stimulus level).[154] For levels 40 to 65 dB SPL, ears with recordable SOAEs produced DPOAEs of statistically significantly greater amplitudes than SOAE-negative ears.[154] For 70 dB SPL the mean DPOAE amplitude was greater in SOAE-positive ears compared with SOAE-negative ears, but the result was not significant.[154] Prieve et al, 1997 also showed that the mean DPOAE levels were higher in SOAE positive ears.[168] Furthermore, Moulin et al (1993) reported that DPOAE amplitudes were significantly larger in ears with SOAEs than those without recordable SOAEs.[130] The DPOAE amplitude has been reported to be higher when recorded at a frequency close to the SOAE frequency, i.e. within a 100 Hz span.[154, 169] Again this increased DPOAE amplitude when recorded close to the frequency of the SOAE has been shown by other researchers.[170] Moulin et al (1993) obtained higher DPOAE amplitudes in the presence of SOAEs, even when ears showing SOAE frequencies less than 300 Hz around DPOAE frequencies were excluded.[130, 154] Thus one would expect that as SOAE-positive ears have DPOAEs and VSOAEs of greater amplitude than SOAE-negative ears, that the relationship between VSOAEs and DPOAEs would be more pronounced when SOAEs are recorded. However, this does not explain the inverse relationship, i.e. decreasing DPOAE levels for increasing VSOAE levels. This inverse relationship may indicate that other factors, as yet unknown, may have played a role. There is a risk that if enough correlation tests are performed, some significant results will occur by chance. However, this does not account for these unexpected experimental findings, as the Bonferroni correction was used.

The results were also shown to be significant for the latter slices VSOAE S_{22}, S_{23} and S_{32}, and this would be expected as the latter slices reflect the nonlinearity of the system more. Once again this does not explain the inverse relationship.

5.5.5) The interaction of VSOAEs, SOAEs, CEOAE I/O functions and DPOAEs

The results demonstrated that there is a significant relationship between both the slices of the VSOAE tested (S_{21} and S_{31}) and CEOAE I/O function for both time windows respectively. The correlation was also significant for the VSOAE S_{31} and DPOAE in the ~1 kHz near octave and approached significance for the VSOAE S_{21} and DPOAE in the ~1 kHz near octave. The correlation approached significance for both the VSOAE S_{21} and S_{31} and the SOAE respectively. As described above these measures all reflect the nonlinearity of the cochlea and are likely to arise from a common cochlear origin. The Volterra slices are related to the nonlinearity of the cochlear input-output function. Presence of SOAEs adds to the nonlinearity. This might be associated with greater distortion and thus DPOAEs, thus the significance of the correlation shown in the ~1 kHz near octave.

CHAPTER 6
DISCUSSION

6.1) Summary of results

Objective 1 was to investigate sex and ear differences with MLSOAEs and how these changed with the stimulus rate. The answer to the latter part of the question is the amplitude of the MLSOAE decreased with increasing stimulus rate, and this result was significant. Female subjects were shown to have MLSOAEs of significantly greater amplitude than male subjects, and right ears produced larger MLSOAEs than left ears. MLSOAEs of greater amplitude were recorded from female right ears compared with female left ears (this difference was significant). There was no significant difference in the MLSOAE amplitudes between male right and left ears.

Objective 2 addressed the sex/side asymmetry with VSOAEs, and also the effect of SOAEs on VSOAEs. SOAEs were found in 66.7% of ears, in agreement with other studies. A higher response amplitude for the VSOAEs was obtained when SOAEs were recorded, and this was found to be highly significant in the cases of the VSOAE S_{21}, VSOAE S_{22} and VSOAE S_{31}. There was also a significant relationship between the rate and the VSOAE amplitude in the presence of SOAEs, with an increased rate resulting in a decreased amplitude response. There was a significant effect of order, with a greater amplitude obtained for the VSOAE second order response compared with the third order response in the presence of SOAEs. The amplitude of the VSOAE response was also significantly greater for the second order slice one, $S_{21,}$ compared with slice two, S_{22}. The amplitude of the VSOAE $S_{21,}$ and S_{22} was found to be significantly greater in female subjects than in male subjects at all rates, when all cases where included (subjects with and without SOAEs). Although VSOAEs $S_{21,}$ S_{22} and S_{31}) obtained from left ears were of greater amplitude than those obtained from right ears (for all responses) ,none of the differences were statistically significant. However the numbers of paired ears compared in these groups was relatively small. For paired responses females were shown to have larger amplitude VSOAE S_{21} obtained from their right ears compared with their left ears; but this finding was not significant. This finding is consistent with the MLSOAE results, except in the case of the MLSOAEs the female right/left asymmetry was found to be significant.

Objective 3 was to investigate how OAEs, reflecting the nonlinearity of the hearing system (SOAEs, I/O function of CEOAEs and DPOAEs), related to one another and most importantly to VSOAEs. There was a significant relationship between the amplitude of the VSOAE and SOAE, reflecting their shared nonlinear origin. There was a significant correlation between the RMS response amplitudes of the VSOAE S_{21} and S_{31} slice waveforms respectively and the slope of the growth function of the first order slice (CEOAE I/O function), thereby reflecting a relationship between the measures of nonlinearity in the amplitude and temporal domains. However there was no significant relationship between the slope of the I/O function and the DPOAE. Furthermore there was no significant relationship between the VSOAEs and DPOAEs in general, except mostly in cases where SOAEs were present. When SOAEs were present, there were significant relationships between the VSOAE S_{22} , VSOAE $S_{23,}$ VSOAE S_{32} and the DPOAE response. This relationship was inverse, however, with a decreasing VSOAE amplitude with an increasing DPOAE. On analysing all correlations of the measures of nonlinearity with one another, there were strong correlations between the CEOAE I/O function and VSOAE S_{21} and S_{31}, indicating a link between nonlinearity in the amplitude and temporal domains. There were also correlations which approached significance between the SOAE and VSOAE S_{21} and S_{31}. There was a significant correlation between the DPOAE in the 1 kHz near octave and the VSOAE S_{31} slice, and the correlation between the DPOAE (~1 kHz) and the VSOAE S_{21} approached significance, suggesting a possible link between nonlinearity in the frequency and temporal domains. Although using linear regression techniques no significant relationships were found in the case of the DPOAEs and VSOAE S_{21} and S_{31} as mentioned above. The nature of any underlying shared physiological mechanisms and shared cochlear sources resulting specifically in these measures of OAE nonlinearity is unknown. The data obtained suggest that the amplitude (CEOAE I/O function) and temporal (VSOAEs) nonlinearities arise from the same generators, whereas the frequency domain nonlinearities (SOAEs & DPOAEs) have different generators.

6.2) Limitations

There were constraints of time for testing and also the length of the study. The research was undertaken in two separate parts; addressing objective 1 and then objectives 2 and 3. This meant that there was some discontinuity between the two experiments, as different ethics approval and machinery were required for both. Time was required to become familiar with the equipment and the recording techniques. The calibration of equipment at the start of experiments also required several days. The experiments were lengthy with an hour being required for each ear, so although subjects were required to sit as still as possible in a reclining, comfortable chair in a sound attenuated booth, there was inevitably some movement and swallowing. This provides a possible explanation for some of the poor correlations obtained between repeat runs.

Subject recruitment was difficult, perhaps due in part to the location of the experiments at The Royal South Hants hospital, and in part to the length of testing. Some funding was available to pay subjects' expenses; however, the majority were volunteers. Male subjects were particularly elusive. Several subjects also failed to meet the inclusion criteria. This made the data analysis more difficult and although we did achieve out target numbers, it was not possible to include all the results due to the poor quality of some responses. Only 'good responses' with repeat waveform correlations of >0.5 in the time window selected were considered to be viable data and were used in the analysis.

6.3) Findings in terms of future applications and clinical applications of the MLS technique

The use of the maximum length sequence technique allows transient evoked otoacoustic emissions to be recorded uncorrupted , at very high stimulus rates, and also allows the extraction of nonlinear temporal interaction components of the system, the VSOAEs.[73, 101] Despite the amplitude of the TEOAE being reduced with this technique, as a very large number of responses are obtained and averaged, the signal-to-noise ratio improves, as does the speed and sensitivity of testing.[101] **Figures 6.0 and 6.1** demonstrate these improvements with the MLS technique, and **table 6.0** shows the results of these advantages.[171] These advantages make the MLS technique desirable when compared with conventional testing. The use of this technique to extract the volterra kernels is also advantageous, as research by this group so far has shown these to be sensitive to cochlear pathology, as manifest by mild hearing impairments.[100]

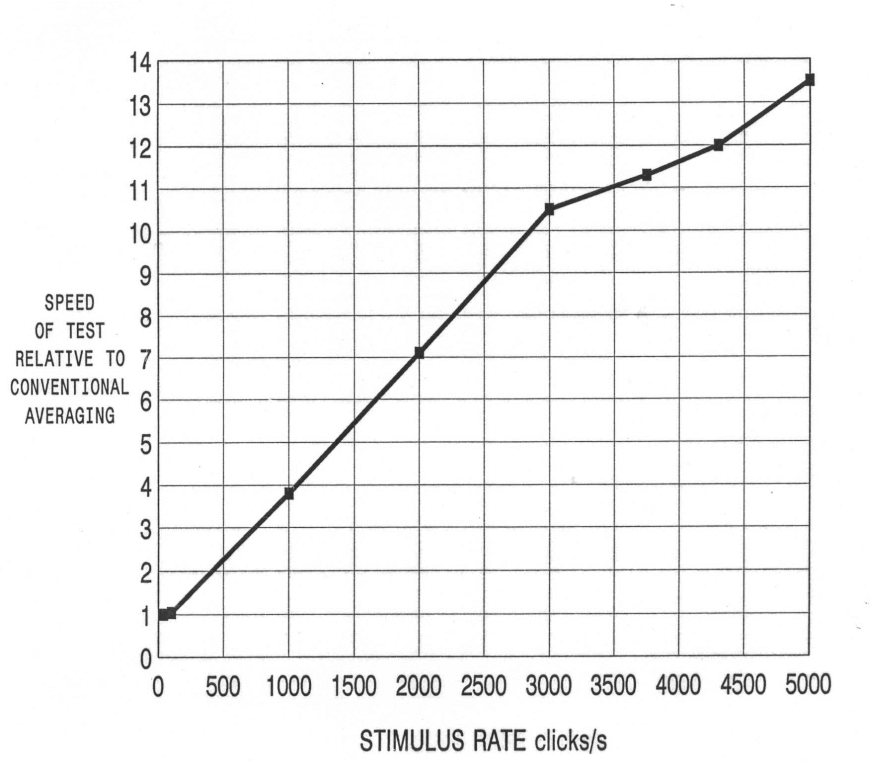


Figure 6.0. *Speed of test relative to conventional methods by stimulus rate.[73]*

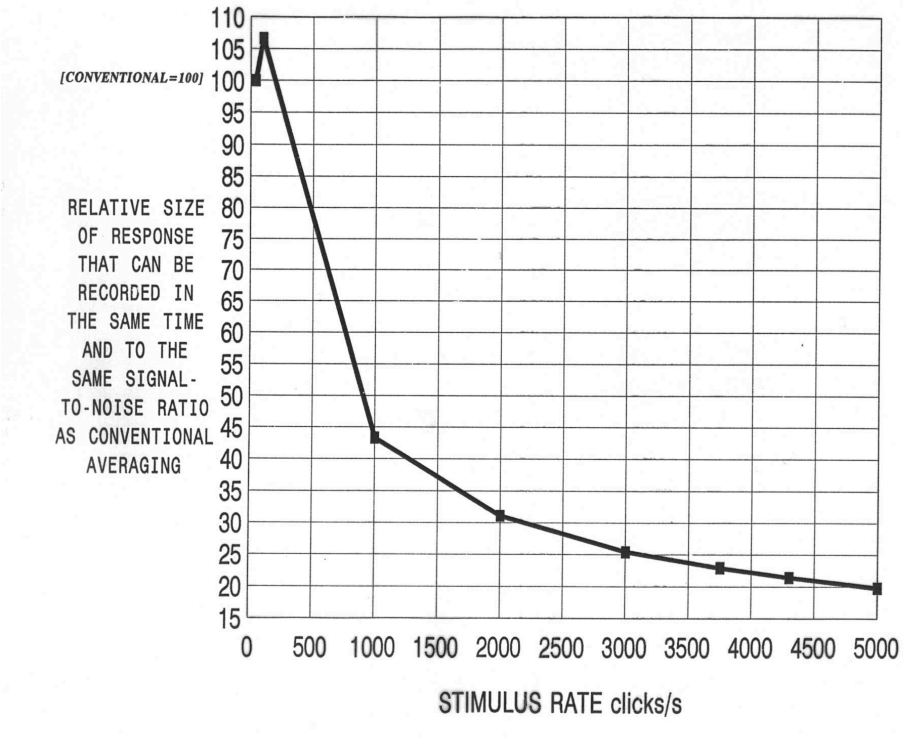


Figure 6.1. *Relative size of the response recorded with MLSOAE at various stimulus rates, for same test duration and to the same SNR, compared with the conventional response.*

	Conventional Response	MLS 5000	MLS 10000
SPEED	100%	1350%	1801%
TIME	100%	7.4% (x13.5)	5.6% (x17.9)
MINIMUM RESPONSE	100%	18%	14%
SIGNAL-NOISE-RATIO	0 dB	11 dB	13 dB

Table 6.0. *Relative data values for the conventional response, MLSs undertaken at a stimulus rate of 5000 clicks/s and a theoretical rate of 10000 click/s. The speed of testing is faster with the MLS technique with a shorter test duration. Lower amplitude responses are detected, with an improved SNR with this technique.*

The problem that has occurred with recording EOAEs with MLS is the noise in the system is the limitation of the SNR.[73] This noise has been identified from three sources.[73] The random noise from the microphone (main contributor), amplifier and analogue digital convertors used means the system at best has a 70 dB SNR, so the signal is only 20 dB above the noise floor (as the requirement for the dynamic range is 92.5 dB).[73] However, this random noise should decrease by averaging. The second source of noise is due to nonlinearities of the system.[73] The third source of noise occurs as a result of incomplete cancellation; as with this technique one click waveform is subtracted from another, therefore the clicks need to be matched precisely in both timing and amplitude.[73]

In this research we have provided normative data for the sex and ear differences that occur with both MLSOAEs and also VSOAEs. The results provided here show that as there is a female/male difference and a right/left asymmetry, then in future studies of these techniques investigating other properties of MLSOAES or VSOAEs ears should be balanced for sex and side. If this is not taken into account differences inaccuracies in interpreting data may arise. The data suggest that as with conventional OAEs there must be a difference, most likely at cochlear level, between females and males, and right and left ears to account for these findings. These sex and ear side differences may possibly be due to a difference occurring in the middle ear, although this is less likely as OAEs arise from the outer hair cells of the cochlea. Thus these sex and ear

differences will impact our future findings when using this technique and will need to be taken into account during data analysis. With regards to the relationship between the nonlinear otoacoustic emissions and VSOAEs, these results are important in helping us to better understand that there is a likely common cochlear origin for the SOAEs, CEOAE I/O function and the nonlinearity in the temporal domain, as reflected by the VSOAEs. As there is a clear correlation between these measures of nonlinearity it is likely that findings that occur with SOAEs and CEOAE I/O function may also apply to VSOAEs. Thus VSOAEs provide a further method for investigating the nonlinear behaviour of the cochlear amplifier.

The use of otoacoustic emissions in the early detection of the effects of noise and also noise induced hearing losses is well established, although few longitudinal studies exist. Thus otoacoustic emission testing would be useful in hearing conservation programmes.[172, 173] Hall and Lutman (1999) studied various methods for early identification of noise induced hearing loss.[78] They evaluated pure tone audiometry, TEOAEs, MLSOAEs and DPOAEs.[78] 'Test-retest reliability was rescaled according to the sensitivity of each measure to differences in hearing threshold level, thus allowing a direct comparison across methods'.[78] The MLSOAE was shown to be the most repeatable method.[78] This was followed by the TEOAE and DPOAE. Thus MLSOAEs have the potential to detect small changes in cochlear function and distinguish these from measurement uncertainties, thus their use in monitoring cochlear function in those exposed to noise and other hazards.[78] As components that may extracted using the MLS technique, VSOAEs may be a more sensitive indicator than MLSOAEs of NIHL, as they reflect the nonlinearity of the system. Indeed in their study of nonlinear properties of otoacoustic emissions in normal and hearing impaired subjects, Thornton et al (2006) showed that MLSOAEs did not differ between the patient and normal groups, but the Volterra slices S_2 and S_3 were significantly altered by even the small degree of hearing loss in the patient group.[86]

de Boer and Thornton (2006) obtained VSOAEs recorded using maximum length sequences from 24 ears of patients with unilateral mild sensorineural hearing losses and compared the results with CEOAEs obtained from the same patients.[174] These results were also compared with the subjects other normally hearing ear.[174] VSOAEs were found to be as effective as CEOAEs at separating normal and hearing impaired ears at the audiometric frequencies of 1 and 2 kHz respectively, moreover at 4 kHz VSOAEs were found to be significantly better as indicators of early sensorineural hearing loss compared with CEOAEs.[174] Thus at the higher frequencies VSOAEs appear to be better at detecting these losses which is desirable as most sensorineural hearing losses commence in the higher frequency ranges.[174] The difference observed was thought to occur as a result of a lack of stimulus artefact contamination of the VSOAEs in the early high frequency portion of the response.[174] Thus the data provided in the current study provide further normative data for VSOAEs that can be used when comparing those of normal hearing with patient/subject groups. The data in the current study also indicate that when comparing two subject groups, the groups must be balanced for sex and ears. However, it must be noted that in de Boers study the comparison was between ears of the same subject.[174]

Click evoked otoacoustic emissions (CEOAEs) have also been recorded from neonates less than 13 hours old, using maximum length sequence stimulation.[175] The high MLS stimulus rates allow the reduction of background noise to occur more quickly, and this improves the Signal to noise ratio (SNR). In this study CEOAES were recorded from 57 ears using a conventional rate (50 clicks/s) and MLS technique (rate of 5000/s).[175] ‘MLS averaging produced an SNR improvement of up to 3.8 dB, with the greatest improvement in higher frequency bands.[175] The improved SNR raised the pass rate between 5-10%. Both SNR and pass rate were lower for 6-10 hours old neonates compared with 10-13 hours old neonates’.[175] Thus the results of this study showed that ‘MLS averaging can reduce false alarm rates by 15% in very young neonates born in hospital’.[175] This has great implications as the failing of a screening test can cause much anxiety and worry in

parents, and having this technique which detects a greater number of valid emissions, will allay some anxieties. In addition this will save the need for repeat testing in the community and therefore NHS time and expenditure. The importance of this finding also illustrates that the use of this technique in the Universal Newborn Hearing Screening programme would be greatly advantageous, in terms of the benefits of greater sensitivity of testing and to some extent speed of testing. The MLS technique, by enabling the recording of both MLSOAEs and extraction of VSOAEs could also prove helpful in the diagnosis of non organic hearing losses and malingerers. The normative data provided by this current research provides good baseline results for the MLSOAEs with large numbers of ears compared with other studies performed by this group, which can be used in future research. The current study also provides normative VSOAE data for 20 pairs of ears (45 ears in total, including unpaired ears), which would be useful in providing baseline results for adult ears.

The use of TEOAEs in children with autism was described in **Chapter 1**.[64] Although TEOAEs were obtained from some of the ears a problem was the test duration, and 10-30 minutes was thought to be required. The reduction in testing time provided by the MLS technique would be of great use in these children and also others who are unable to keep still for longer periods of time.

OAEs have been performed in patients with ototoxicity. Stavroulaki et al (2002) studied whether TEOAEs or DPOAEs were more sensitive than pure tone audiometry in revealing Gentamicin ototoxicity in children with cystic fibrosis.[176] The subjects in the study were a cohort of 12 children with cystic fibrosis with normal hearing thresholds, and a history of gentamicin exposure.[176] Two control groups of similar aged children were used; one of 8 children with cystic fibrosis and no gentamicin exposure and one of healthy volunteers were also included. The pure tone audiogram findings were normal for all groups.[176] The cystic fibrosis group treated with gentamicin had lower TEOAE levels and DPOAE amplitudes, with a further decrease after the administration of the gentamicin. DPOAEs were found to be the most sensitive measure.[176] This same group also measured DPOAEs in patients

undergoing haemodialysis and found no change before and after dialysis, although approximately 55% of dialysis patients had sensorineural hearing losses of unknown aetiology.[177] Thus MLSOAEs and also VSOAEs could be used in these types of cases, and supersede the use of conventional emission testing in cases such as these as they are more sensitive to changes in the cochlea. As mentioned before the MLS technique enables the detection of changes that are missed by conventional OAE testing, and the VSOAEs are more sensitive at identifying early losses.[100] This suggestion is supported by Hall and Lutmans' (1999) study the findings of which, suggest that MLSOAEs are likely to be the more sensitive indicator of early loss than conventional emissions, as ototoxic drugs usually affect the cochlear outer hair cells.[78] VSOAEs are more sensitive than MLSOAEs in the detection of mild hearing losses so may provide a better tool when ototoxic agents are used for example the chemotherapeutic agent Cisplatin. With increasing survival rates prevention and /or early detection of ototoxicity are important in providing management options.[178] This may help medical practitioners to decide on the dosage required. The normative data provided in this current study could be used in future studies to compare with MLSOAEs and VSOAEs obtained from patients given ototoxic drugs. The VSOAEs, based on previous findings may prove to be the most sensitive indicators of early losses caused by ototoxic drug exposure.[86]

In the follow up of dynamic pathologies MLSOAEs and VSOAEs may prove a very useful tool. Examples are in Meniere's disease, and also evaluating responses to various treatments. The effects of aging and MLSOAEs and VSOAEs could also be studied. Furthermore early detection of later onset familial type hearing losses using the VSOAEs obtained using the MLS technique would be greatly advantageous in terms of advice given to patients and hearing conservation. This highlights only a few of the potential uses for this technique. Once again the normative data provided for MLSOAEs and VSOAEs in the current study could be used for comparison in these potential future studies.

MLSOAEs would also provide a useful indicator of whether a loss is cochlear or retrocochlear. Thus if the patient had a hearing loss and normal MLSOAE the hearing loss would be more likely to be retrocochlear, therefore the patient could be referred on for MRI scanning. If the MLSOAE was abnormal the loss would likely to be cochlear, therefore saving the money and also a space on the list required for an MRI scan. The condition of auditory neuropathy is the exception to this rule. VSOAEs could also play a role in the differential diagnosis of peripheral end organ and neural lesions, 'as the types of nonlinearity produced by these two conditions may be quite different'.[87] Deltenre et al (1997) showed that evoked potentials, in combination with OAEs, can provide a powerful tool in the diagnosis of difficult cases. [87, 179]

The potential monitoring of cochlear function during vestibular schwannoma surgery could potentially be undertaken more efficiently using the MLS technique. The reduced recording time obtained using this technique may be of great benefit, shortening the time between surgical intervention and the observation of the resultant effect.[87]

MLSOAEs and VSOAEs are likely to prove to be very useful tools in clinical practise. The study by de Boer et al (2007) on neonates taps into only a fraction of the potential uses of this system. Here we have provided some normative data for both sexes and ears for the MLSOAE and VSOAE measures, and also investigated how the VSOAEs relate to other measures of nonlinearity. More research is needed to investigate why these sex and ear differences occur. With regards to the investigation of the relationship between VSOAEs and DPOAEs further research is needed, perhaps looking at a broader frequency range, looking at the I/O function of DPOAEs, or a different product (i.e. 2f2-f1 instead of 2f1-f2) to see if there is any direct relationship between these two measures of nonlinearity.

Research is required into all the potential uses of this innovative technique and the results obtained in future studies can be compared to those obtained from the groups of normals investigated in this current study. Further refinement of the equipment required, and providing more user friendly systems that would work better in a clinical setting is necessary. It is hoped that this technique will prove to be an invaluable tool in medical practice in the future.

APPENDIX 1

SUBJECT INFORMATION SHEETS AND CONSENT FORM

MRC
Medical Research Council

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Royal South Hants Hospital, Southampton
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Hospital Line (023) 8082 5310
Email: Hasnaa@soton.ac.uk

LREC Submission Number: 105/01

Ear and sex differences for Maximum Length Sequence otoacoustic emissions

(MLSOAEs),

in normally hearing adult subjects

Patient Information Sheet

You are being invited to take part in a research study investigating the differences in Otoacoustic Emissions (OAE) recorded from men and women, left and right ears. Before you decide to participate in the study it is important for you to understand why the research is being done and what it will involve. Please read the following information carefully and do not hesitate to ask any questions you may have.

Otoacoustic emissions (OAE) are sounds produced by the hearing organ (cochlea) of normally hearing ears in response to acoustic stimuli. This response can be recorded by placing a small insert containing a very sensitive microphone in the outer ear canal. Many hospitals use this test to check that new-born babies can hear. The Institute of Hearing Research in Southampton have developed a new OAE technique (called MLSOAE) which allows you to increase the rate at which the acoustic stimuli are delivered and means that you can record in a shorter time than conventionally or record smaller responses by recording for the same time.

It has been previously demonstrated that conventional OAEs differ between ears and the sexes. Before we can use MLSOAE in clinical practice, it is necessary to establish if there are any differences in the MLSOAE between ears and the sexes. Using the data we collect, we hope to find out more about how the ear processes the sounds it receives and may allow us to improve screening for hearing loss and the diagnostic evaluation of hearing disorders.
The experiment will begin with a few very routine hearing tests. Firstly, the researcher will ask some questions about your hearing and then examine your ear canals to check they are clear. The state of your ear drum and middle ear will then be assessed by a very simple test

known as tympanometry (this involves placing a small probe in the entrance of your ear canal and measuring the ear's response to slight changes in air pressure).

For the final test, all you will need to do is to relax in a comfortable chair and stay as still as possible. The researcher will place another small insert at the entrance of your ear canal. You will hear clicking or buzzing sounds, and the response from your ear (which is itself a very quiet sound) will be recorded.

The whole session is expected to take no more than one hour per ear tested.

The Southampton and South West Hants Local Research Ethics Committee has reviewed and approved this study. All information obtained will remain confidential and be kept in accordance with the Data Protection Act. You have the right to withdraw from this study at *any* time without giving a reason.

If you require any further information please do not hesitate to contact either Miss Hasnaa Ismail or Dr Toni Slaven at the Institute of Hearing Research, Royal South Hants Hospital, Southampton on (023) 8063 9746 or via email on A.Slaven@soton.ac.uk.

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Patient Information Sheet

LREC Submission Number: 264/03/w

Patient identification Number:……

Study title: The relationship between non linear properties of various types of otoacoustic emissions (OAEs).

You are being invited to take part in a research study. Before you decide to participate in the study it is important for you to understand why the research is being done and what it will involve. Please read the following information carefully and do not hesitate to ask any questions you may have.

The purpose of this study:
Otoacoustic emissions (OAE) are sounds produced by normally hearing ears, recorded by placing a small probe in the outer ear canal. They are considered to reflect the clinical status of the inner ear and are used in many hospitals to test the hearing of new-born babies.

This study aims to describe and compare some characteristic properties of various types of OAEs obtained from normally hearing ears. These include a novel type of OAE test that has recently been developed at the Medical Research Council, Institute of Hearing Research, Southampton. Using the data we collect, we hope to find out more about how the ear processes the sounds it receives and the way in which OAEs reflect this process. This may allow us to improve the tools used in screening for hearing loss in newborns and in the diagnostic evaluation of hearing disorders.

Subjects:
We are looking for healthy volunteers with normal hearing. If you are found to have normal hearing on standard hearing tests you will be asked to undertake the tests involved in the study. We are aiming to test 50 ears, but we may need to test more subjects depending what the experiment shows.

Your involvement in the experiment:
The experiment will begin with a few very routine hearing tests. Firstly, the researcher will ask some questions about your hearing and then examine your ear canals to check they are

clear. The state of your ear drum and middle ear will then be assessed by a very simple test known as tympanometry (this involves placing a small probe in the entrance of your ear canal and measuring the ear's response to slight changes in air pressure). You will then undergo a standard hearing test. These tests should take about 30 minutes to complete. If we find that your hearing is not within the norms for your age group, you will be informed and no further testing undertaken.

Procedure:
For the final tests, all you will need to do is to relax in a comfortable chair and stay as still as possible. The researcher will place another small insert at the entrance of your ear canal. You will hear clicking or buzzing sounds, and the response from your ear (which is itself a very quiet sound) will be recorded.

The whole session is expected to take no more than one hour. You may be requested to attend a session at a later date to test the other ear.

Risks and benefits:
This equipment has been used for routine diagnosis and as a research tool in many hospitals around the world. The sound level that will be used for the test should not affect your hearing during or after the test. If a previously unknown hearing loss is discovered through routine tests then, with your consent, we have a 'duty of care' follow-up procedure which involves sending a letter to your GP informing him/her of the result. Any such discovery will terminate your participation in the project. If we have to stop the experiment for any other reasons, we will explain the reasons why and you may be asked to return for another session. This project will be of no direct benefit to you. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. You have the right to withdraw from this study at *any* time without giving a reason.

Confidentiality and publication:
All information obtained will remain confidential and be kept in accordance with the Data Protection Act. Any information about you that leaves the hospital will have your name and address removed so that you cannot be recognized from it. The data collected will be published in a scientific journal once all the subjects have been tested. It may also be presented at meetings. You can be assured that your name will not appear on any written or oral communications.

Funding, approval and complaints:
The South & West Local Research Ethics Committee has approved the study. If taking part in this research project harms you, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will be available to you.

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MRC
Medical Research Council

LREC Submission Number: 105/ 01

Patient Identification Number: ……

Patient Consent Form

Title of Project: Ear and sex differences for Maximum Length Sequence otoacoustic emissions (MLSOAEs), in normally hearing adult subjects
Name of Researcher: Miss H. Ismail, Professor R. Thornton

Please initial box

1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without my medical care or legal rights being affected. ☐

3. I agree to take part in the above study. ☐

Name of Patient	Date	Signature

Name of Person taking consent (If different from researcher)	Date	Signature

Researcher	Date	Signature

Thank you very much for taking part in this study.

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MRC
Medical Research Council

LREC Submission Number: 264/03/w
Patient Identification Number: ……
Patient Consent Form

Title of Project: Investigating the Properties of Various Types of Otoacoustic Emissions
Name of Researcher: Miss H. Ismail, Professor R. Thornton

Please initial box

4. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions. ☐

5. I understand that my participation is voluntary and that I am free to withdraw at any time, without my medical care or legal rights being affected. ☐

6. I agree to take part in the above study. ☐

Name of Patient	Date	Signature

Name of Person taking consent (If different from researcher)	Date	Signature

Researcher	Date	Signature

Thank you very much for taking part in this study.

APPENDIX 2
QUESTIONNAIRES

MRC INSTITUTE OF HEARING RESEARCH
Royal South Hants Hospital, Southampton
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MRC
Medical Research Council

LREC Submission Number: 105/01

Ear and sex differences for Maximum Length Sequence otoacoustic emissions (MLSOAEs), in normally hearing adult subjects

QUESTIONNAIRE

(To be administered by researcher)

Patient Identification Number: …….

Sex: ……

DOB/ Age: …….

1. Do you have a hearing loss? **YES/NO**
   If yes, in which ear?
   LEFT/RIGHT/BOTH
2. Have you had a hearing test before **YES/NO**
   YEAR……
3. Have you ever had:
   An ear infection **YES/NO L/R**
   YEAR……
   Ear discharge **YES/NO L/R YEAR……**
   Ear operation **YES/NO L/R YEAR……**
4. Do you suffer from tinnitus?(Ringing in ear/s)
   YES/NO
   (Episodes lasting longer than 5 minutes)
   In which ear? **L/R/BOTH/IN HEAD**
   What does it sound like?
   RING/HUM/WHISTLE/BUZZ/HISS/OTHER

If 'OTHER' please state: **…….**

5. Have you been exposed to very loud noise e.g Gunfire or Music? **YES/NO**
   Do you think it has affected your hearing? **YES/NO**
   Have you been exposed to a loud noise just prior to coming here? **YES/NO**

6. Have you suffered from a cold recently? **YES/NO**

7. View of left eardrum: **CLEAR/PARTIALLY OCCLUDED/COMPLETELY OCCLUDED**

8. View of right eardrum: **CLEAR/PARTIALLY OCCLUDED/COMPLETELY OCCLUDED**

ADDITIONAL INFORMATION

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MRC
Medical Research Council

LREC Submission Number: 264/03/w

The relationship between nonlinearities in Distortion Product Otoacoustic Emissions (DPOAE's), Spontaneous Otoacoustic Emissions (SOAE's), Volterra kernels and Input/Output (I/O) function
In normally hearing adult subjects

QUESTIONNAIRE

(To be administered by researcher)

Patient Identification Number: …….

Sex: ……

DOB/ Age: …….

9. Do you have a hearing loss? **YES/NO**
   If yes, in which ear?
   LEFT/RIGHT/BOTH

10. Have you had a hearing test before **YES/NO**
    YEAR……

11. Have you ever had:
    An ear infection **YES/NO** **L/R**
    YEAR……

    Ear discharge **YES/NO** **L/R**
    YEAR……

    Ear operation **YES/NO** **L/R**
    YEAR……

12. Do you suffer from tinnitus?(Ringing in ear/s)
    YES/NO
    (Episodes lasting longer than 5 minutes)

    In which ear? **L/R/BOTH/IN HEAD**

    What does it sound like?
    RING/HUM/WHISTLE/BUZZ/HISS/OTHER

If 'OTHER' please state:

…….

13. Have you been exposed to very loud noise e.g. Gunfire or Music? **YES/NO**
Do you think it has affected your hearing? **YES/NO**

Have you been exposed to a loud noise just prior to coming here? **YES/NO**

14. Have you suffered from a cold recently? **YES/NO**

15. View of left eardrum: **CLEAR/PARTIALLY OCCLUDED/COMPLETELY OCCLUDED**

16. View of right eardrum: **CLEAR/PARTIALLY OCCLUDED/COMPLETELY OCCLUDED**

ADDITIONAL INFORMATION

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QUESTIONNAIRE

(To be administered by researcher)

Patient Identification Number: …….

Sex: ……

DOB/ Age: …….

17. Do you have a hearing loss? **YES/NO**
    If yes, in which ear?
    LEFT/RIGHT/BOTH

18. Have you had a hearing test before **YES/NO**
    YEAR……

19. Have you ever had:
    An ear infection **YES/NO** **L/R**
YEAR……
    Ear discharge **YES/NO** **L/R**
YEAR……
    Ear operation **YES/NO** **L/R**
YEAR……

20. Do you suffer from tinnitus?(Ringing in ear/s)
    YES/NO
    (Episodes lasting longer than 5 minutes)
    In which ear? **L/R/BOTH/**
    IN HEAD

What does it sound like?
RING/HUM/WHISTLE/BUZZ/HISS/OTHER

If 'OTHER' please state:
…….

21. Have you been exposed to very loud noise e.g. Gunfire or Music? **YES/NO**
Do you think it has affected your hearing? **YES/NO**
Have you been exposed to a loud noise just prior to coming here? **YES/NO**

22. Have you suffered from a cold recently? **YES/NO**

23. View of left eardrum: **CLEAR/PARTIALLY OCCLUDED/COMPLETELY OCCLUDED**

24. View of right eardrum: **CLEAR/PARTIALLY OCCLUDED/COMPLETELY OCCLUDED**

ADDITIONAL INFORMATION

APPENDIX 3
DATA COLLECTION FORMS

MLS OTOACOUSTIC EMISSIONS DIFFERENCES BETWEEN SEX AND EARS OF NORMALLY HEARING ADULTS (Study No 105/01)

Patient Identification No: …… Date: / /200
Ear Lt / Rt
Click Amplitude
Click in ear canal recorded at 70dB, 40 clicks/s (order 1) and for 80 clicks.

MLS File Naming Protocol

Data Filename: aabcd where

aa = subject number (01 to 99)
b = ear and sex (1 for left male 2 for left female 3 for right male 4 for right female)
c = rate (1 for 40, 2 for 300, 3 for 500, 4 for 1000, 5 for 2000, 6 for 3000, 7 for 4282, 8 for 5000/s)
d = level (6 for 60dB, 7 for 70dB)

60dB

Filename	Rate	Order	No of MLS	Order Presented
_ _ _16	40	1	1200	
_ _ _16	40	1	1200	
_ _ _26	300	10	8	
_ _ _26	300	10	8	
_ _ _36	500	10	14	
_ _ _36	500	10	14	
_ _ _46	1000	10	28	
_ _ _46	1000	10	28	
_ _ _56	2000	10	59	
_ _ _56	2000	10	59	
_ _ _66	3000	10	88	
_ _ _66	3000	10	88	
_ _ _76	4282	10	120	
_ _ _76	4282	10	120	
_ _ _86	5000	10	141	
_ _ _86	5000	10	141	

70dB

Filename	Rate	Order	No of Clicks	Order Presented
_ _ _17	40	1	1200	
_ _ _17	40	1	1200	
_ _ _27	300	10	8	
_ _ _27	300	10	8	
_ _ _37	500	10	14	
_ _ _37	500	10	14	
_ _ _47	1000	10	28	
_ _ _47	1000	10	28	
_ _ _57	2000	10	59	
_ _ _57	2000	10	59	
_ _ _67	3000	10	88	
_ _ _67	3000	10	88	
_ _ _77	4282	10	120	
_ _ _77	4282	10	120	
_ _ _87	5000	10	141	
_ _ _87	5000	10	141	

RELATIONSHIP BETWEEN NONLINEARITIES IN DPOAE's, SOAE's, VOLTERRA KERNELS AND I/O FUNCTION IN EARS OF NORMALLY HEARING ADULTS

(Study No 264/03/w)

Patient Identification No: …… Date: / /200
Ear Lt / Rt

File Naming Protocol

Data Filename for SOAE: aabcd where

aa = subject number (01 to 50)
b = ear (1 for right, 2 for left)
c = sex (1 for female, 2 for male)
d= SOAE (s at end of filename to identify as SOAE file, **s** only for first runs, **sn** at end for second runs)

Data Filename for DPOAE: aabcd where

aa = subject number (01 to 50)
b = ear (1 for right, 2 for left)
c = sex (1 for female, 2 for male)
d= DPOAE (i.e. d for DPOAE)

Data Filename for VK: aabcd where

aa = subject number (01 to 50)
b = ear (1 for right, 2 for left)
c = sex (1 for female, 2 for male)
d = rate for VK (1vk for 800/s, 2vk for 1000/s, 3vk for 1200/s, stimulus level 70dB for both (vk to identify as VK file))

Data Filename for I/O function: aabcde where

aa = subject number (01 to 50)
b = ear (1 for right, 2 for left)
c = sex (1 for female, 2 for male)
d= stimulus level for I/O function (40 for 40dB, 50 for 50dB, 60 for 60dB and 70 for 70dB)
e= run (**1** for first run, **2** for second run)

SOAE
(First two runs)

Filename	Done
_ _ _ _ s	
_ _ _ _ s	

DPOAE

Fixed frequency levels 73 dBHl for f1
And 67 dBHl for f2
Sweep between 750kHz and 4kHz (setup filename dp750-4)

Filename	Done
_ _ _ _ d	
_ _ _ _ d	

SOAE
(Second two runs)

Filename	Done
_ _ _ _ sn	
_ _ _ _ sn	

Volterra Kernels

(Rates presented in reverse order for alternate subjects, 1vk for 800/s, 2vk for 1000/s, 3vk for 1200/s stimulus level 70 dB for both)

Filename	Done
_ _ _ _ 1vk	
_ _ _ _ 1vk	
_ _ _ _ 2vk	
_ _ _ _ 2vk	
_ _ _ _ 3vk	
_ _ _ _ 3vk	

I/O Function

(Measured at conventional click rate 40/s presented in order 40, 50, 60, 70 then reverse order for second run, i.e. 70, 60, 50, 40)

Filename	Stimulus level	Done
_ _ _ _ 401	40	
_ _ _ _ 402	40	
_ _ _ _ 501	50	
_ _ _ _ 502	50	
_ _ _ _ 601	60	
_ _ _ _ 602	60	
_ _ _ _ 701	70	
_ _ _ _ 702	70	

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